ASSOCIATION OF DENTAL PLAQUE, POOR ORAL HYGIENE AND PERIODONTAL DISEASE WITH HELICOBACTER PYLORI INFECTION

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Certificate

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ABSTRACT

Aim:

Peptic ulcer, the most common stomach disease is now well accepted as an infectious disease; and the causative agent H. pylori must be treated with antibiotics. Recently attention has been focused on the importance of dental plaque in harbouring H.pylori and its role as a potential reservoir for gastric infection & reinfection. This study was undertaken to determine whether dental plaque harbours H. pylori and to determine the association between oral hygiene and periodontal disease status and H.pylori gastritis

Materials and Methods:

110 patients with dyspeptic symptoms and clinical indications for an upper gastroendoscopy from the Department of Medical Gastroenterology, Government General Hospital Chennai were selected for the study. Oral hygiene status and periodontal disease status were examined and study variables were obtained from patient's history. Among the 110 patients, 55 patients who had a positive H.pylori serology or positive rapid urease test or histologic evidence for the presence of H.pylori in antral biopsy specimens were categorized as cases. The remaining 55 patients who were negative for these tests were controls. The presence of H.pylori in dental plaque was detected by the rapid urease test and culture.

Results:

It was found that the association of periodontal disease and poor oral hygiene with H.pylori gastritis was not significant. The RUT was positive in 87.3% of cases and 52.7% of controls. But there was no cultural detection of H. pylori in dental plaque.

Conclusion:

Hence the presence of H.pylori in dental plaque is inconclusive. Further studies using larger sample size and specific methods of detection of H.pylori are required to better assess the role of dental plaque as a reservoir for H.pylori and its relationship with H.pylori infection.

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LIST OF ABBREVIATIONS

1. AAP	–	American Academy of Periodontology
2. BOD	–	Biological oxygen demand
3. CAL	–	Clinical attachment level
4. CEJ	–	Cemento Enamel Junction
5. CI-S	–	Calculus Index- Simplified
6. CLO	–	Campylo-bacter like organism
7. DI-S	–	Debris Index- Simplified
8. ELISA	–	Enzyme linked immunosorbent assay
9. H. pylori	–	Helicobacter pylori
10. IgG	–	Immunoglobulin G
11. MALT	–	Mucosa associated lymphoid tissue
12. NHANES	–	National Health and Nutrition Examination Survey
13. NSAIDs	–	Non-steroidal anti-inflammatory drugs
14. OHI-S	–	Oral hygiene index- Simplified
15. PPD	–	Probing pocket depth
16. RU/ml	–	Relative units/ml
17. RUT	–	Rapid urease test
18. RUT(P)	–	Rapid urease test(plaque)
19. RUT(B)	–	Rapid urease test(biopsy)
20. SES	–	Socioeconomic status
21. SDS	–	Sodium dodecyl sulphate

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INTRODUCTION

The stomach contains about 10^4 bacterial cells/ml of gastric content (***Marianne Quiding – Jabrink et al 2009)***.[67] The comparatively low number of bacteria in the stomach results from the low pH (pH-2) caused by the production of hydrochloric acid and a considerable flow (the turnover rate is 4-6 l/hr) (***Tannock 1995)***.[104] Until recently, the stomach was considered to be without an established normal flora. H.pylori, a spiral shaped, motile gram – negative, micro – aerophilic bacterium was then discovered (***Marshall BJ, Warren JR 1984)***.[70]

It is now well accepted that the most common stomach disease, peptic ulcer is an infectious disease and that the causative agent, H. pylori must be treated with antibiotics (***European Study Group 1997)***.[31] Stomach infection with this organism causes inflammation of the gastric mucosa which can lead to gastritis, duodenal or gastric ulcers and even in rare cases to gastric carcinoma or MALT (mucosa associated lymphoid tissue) lymphoma (***Czenikiewicz- Guzik et al 2004)***.[22]

Helicobacter pylori is one of the most common bacterial infections in humans (***Blaser 1997)***.[15] Approximately 50% of the world's population is believed to be infected with H.pylori (***Czenikiewicz- Guzik et al 2004).***[22] Serological tests have shown that the carriage rate of H.pylori is reported to be 20 – 80% for adults in the developed world and this figure may rise to more than 90% in the developing world ***(Taylor & Blaser 1997)***.[105] The majority of infected individuals do not develop clinically apparent disease, but there is now indisputable evidence that 6-20% of infection results in peptic ulceration and a smaller proportion (less than 1%) are associated with gastric cancer. Infection in a given individual will result either in the peptic ulcer pathway with associated increased acid output or the chronic atrophic

gastritis – carcinoma pathway which is associated with hypo or achlorhydria (***Michael G. Farthing 1998)***.[79]

Although eradication of H.pylori can be achieved with a combination therapy of antibiotics, the possibility of recurrence is very high (***Unge P 1996)***.[107] The reservoir of H. pylori and its mode of transmission are unclear. A faecal- oral, oral-oral and gastro- oral route of infection have been suggested (***Mai et al 1989)***.[61] Recently researchers have suggested that the primary extra gastric reservoir for H.pylori is the oral cavity (***Nabwera, Logan 1999)***.[84] H.pylori has been detected by various methods in dental plaque, [49,62,25,7,52,65] which has led to the suggestion that dental plaque may be responsible for the transmission of the bacteria and possibly serve as a source of reinfection after eradication treatment. However some studies[21, 22, 94] have reported no correlation between dental presentation of the micro organism and H.pylori associated gastritis. The hypothesis that oral flora may be a permanent reservoir of viable H.pylori is still inconclusive.

Since human infection by this pathogen appears to involve an oral route, it seems biologically plausible that oral health status directly or indirectly influences the process of H.pylori infection or reinfection.

This study was undertaken (a) to determine whether dental plaque harbors H.pylori and may act as a reservoir for the organism and (b) to determine the relation between H.pylori gastritis and dental health.

AIM AND OBJECTIVES

The aim of the present study was to determine whether dental plaque harbors H. pylori and thereby act as a reservoir for the organism and to determine the relation between oral hygiene status and periodontal disease status with H. pylori infection.

For this purpose the following objectives were undertaken:

1. To determine the presence of H. pylori in dental plaque of patients with dyspeptic symptoms and indications for gastroendoscopy

2. To determine the presence of H. pylori in dental plaque of patients with good, fair and poor oral hygiene and correlate with H. pylori in antral biopsy of the same patients.

3. To determine the presence of H. pylori in periodontitis patients and correlate with H. pylori infection.

REVIEW OF LITERATURE

The discovery of Helicobacter pylori in 1982 by ***Warren and Marshall 1983***[112] was the starting point of a revolution concerning the concepts and management of gastro duodenal disease. The public health importance of H.pylori and its role in stomach diseases was recognized in 2005 by the attribution of the Nobel Prize in Physiology or Medicine to **B.Marshall** and **R.Warren**. In the history of Noble Prizes, this is only the third time that the discovery of a bacterium has been acknowledged.[35]

PRE H. PYLORI ERA

Donati M. 1586[26] gave the first description of gastric ulceration.

Morgagm G.B. 1761[82] described erosions and erythema of the stomach and duodenum in patients with heartburn and upper abdominal pain.

Billroth.CAT. 1881[11] described gastric surgeries- gastroduodenostomy and gastrojejunostomy.

Von Mikulicz – Radecki J. 1881[111] reported the first potentially usable endoscope.

Schwarz K. 1910[100] gave the famous dictum “no acid, no ulcer”

Sippy B.W. et al 1915[101] recommended antacids in the treatment of peptic ulcer.

Ash. ASF. Schild H.O. 1966[3] described the gastric histamine receptor.

Black J.W. et al 1972[14] reported the first H_2 receptor antagonist.

Ganser A.L., Forte J.G. 1973[37] demonstrated the presence of a potassium stimulated adenosine triphosphate pump. Six years later the first commercially available proton pump inhibitor was reported.

KEY DATES IN HISTORY OF H. PYLORI

1893[13] - Gastric spiral bacteria were reported for the first time in the stomach of dogs

1906[53] - Spirochetes were demonstrated in the human stomach

1924[58] - Urease activity in the stomach was reported

1950[33] - Urease in patients with gastric ulceration was reported to neutralize gastric acid via the production of ammonia.

1954[92] - A study of 1000 gastric biopsy specimens failed to confirm the presence of bacteria

1975[103] - Gastric spirochetes and gastritis were present in 80% of gastric ulcers

1983[112] - **Discovery of H. Pylori**. Campylobacter – like organisms associated with gastritis and possibly peptic ulceration – beginning of modern era. This was first presented at the Royal Australian College of Physicians on 22nd October 1982 and published in letter form in 1983.

1985[69] - Temporal relationship was established between acquisition of H.pylori and development of gastritis.

1987[20] - It was proposed that eradication of H.pylori leads to long – term cure of duodenal ulceration

1989[40,30] - The genus 'Helicobacter' was suggested.

1994[46] - H.pylori was classified as a grade I (definite carcinogen)

1994[88] - It was suggested that the infection should be eradicated in patients with peptic ulcers.

1997[64] - European consensus report was given on the management of H.pylori infection.

EPIDEMIOLOGY OF H.PYLORI

H.pylori has been reported world wide, but no common extra human reservoir of H.pylori has been detected. Although prevalence is decreasing, H.pylori infection remains one of the most common in man.

Roger A. Feldman et al 1988[99] after analysis of data of several studies reported that the major period of H.pylori acquisition is in childhood.

Mendall et al 1992[78] found that crowded living conditions are also associated with increased carriage rates of H.pylori.

The Eurogast study group 1993[106]***, Gasbarrini G. et al 1995***[38]reported that the seroprevalence of H.pylori infection is often similar in males and females.

Fox JG. 1995[34] reported that cats are found with H.pylori in nature and could be a reservoir for human infection.

Isabelle. M. Madinier et al 1997[47] found that in developing countries, almost all children are infected by age 10. The major risk factors are poor sanitary conditions during childhood.

Malaty.H.M. et al 1996[63], ***the Eurogast Study group 1993***[106], ***Veldhuyzen van Santen S.J. 1995***[110] in their studies found that lower socio – economic status and/or a low level of education are associated with an increase in prevalence of H.pylori infection.

Roger A. Feldman et al 1998[99] reported that consumption of alcohol associated with seropositivity, may be an indirect measure of socioeconomic and cultural variables.

Dowsett S.A., M.J. Kowolik 2003[27] reported that once H.pylori is acquired, it persists into old age, unless eradicated by treatment.

H.PYLORI IN DENTAL PLAQUE – ORAL CAVITY AS A RESERVOIR FOR H.PYLORI

The human stomach had long been considered a sterile organ unsuitable for microbial colonization because of its acidity

Marshall FJ. et al 1984[72], ***Marshall FJ et al 1987***[71], ***Goodwin CS et al 1989***[40] demonstrated that Helicobacter pylori was a gram – negative microaerophilic motile bacterium, especially adapted to life in the human stomach.

Megraud F et al 1985[77] found that H. pylori was capable of surviving and proliferating in this hostile environment by mobility in the gastric mucus (flagella, enzymatic mucus degradation), partial neutralization of gastric

acidity (urease converting urea into ammonia), and specific adherence to gastric epithelial cells.

Marshall et al 1985[69] reported that the mode of transmission of H.pylori remains something of a mystery. Since blood borne infection seems improbable, H.pylori must reach the stomach, its primary residence, via the oral cavity. ***Marshall (1985)***, [69] in an attempt to convince the then many skeptics of the role of H.pylori in gastric disease, ingested H.pylori orally to confirm that it could indeed cause gastritis. He developed symptomatic gastritis thereby fulfilling Koch's postulates

Dowsett S.A., Kowolik. M.J. 2003[27] reported that to date, there has been little success in identifying a consistent non human source of infection, and it was likely that H.pylori is spread directly from person to person.

DETECTION OF H.PYLORI FROM ORAL SPECIMENS

Studies have shown frequent isolation of H.pylori from oral specimens particularly dental plaque.

H.pylori has been detected in subgingival plaque ***Pustorino et al 1996***[95], ***Dowsett et al 1999***[28], ***Riggio and Lennon 1999,***[98] saliva and oral lesions ***Mravak – Stipetic et al 1998***[83], ***Birek et al 1999*** [12] on oral mucosa ***Mravak – Stipetic et al 1998***[83], ***Dowsett et al 1999***[28], ***Allaker et al 2002,***[1] and in supragingival plaque ***Allaker et al 2002***[1], ***Kim et al 2000***[51], ***Song et al 2000.***[102]

Different methods have been used for the diagnosis of gastric infection and for the detection of oral H. pylori.

RAPID UREASE TEST

Gill et al 1994[36]***, Pytko – Polonczyk et al 1996***[96]***, Desai et al 1998***[25]***, Butt et al 1999***[17]***, and Avcu et al 2001***[5]***, Pradeep et al 2006***[94] – These studies used the rapid urease test for the detection of oral H.pylori.

H.pylori is the only urease positive bacterium known to reside in the stomach; hence the rapid urease test provides a suitable detection method for use on gastric samples. The oral cavity is residence to several urease producing specimens including Streptococcus species, Haemophilus species and Actinomyces species. ***Vaira et al 1998***[109] reported that the urease producing oral bacteria unlike H.pylori usually cannot give positive results within an hour.

CULTURE

Krajden et al 1989[52] reported first the presence of H.pylori in oral cavity, when the bacterium was cultured from the dental plaque of one of 29 patients with H.pylori associated gastric disease. Since then culture of oral H.pylori has met with limited success.

The detection rates by culture in dental plaque samples have been consistently low. There are few exception studies.

Majumdar et al 1990[62] had oral H.pylori culture positive samples in 40/40 patients and ***D'Alessandro and Seri 1992***[23] isolated H.pylori in 16/ 20 patients by culture from dental plaque.

Oshowo et al 1998[90] isolated H.pylori by culture from dental plaque in 2 of 180 patients.

Parsonnet et al 1999[93] obtained 3 culture positive samples from a total of 26 patients.

Namavar et al 2001[85] – In both the studies with high culture positive results, confirmation of isolate identity by means of molecular methods was not performed and as a result there is a potential for false – positives.

Allaker et al 2002[1]***, Luman et al 1996***[59]***, Hardo et al 1995***[44] could not isolate H.pylori by culture from dental plaque - 0/109, 0/100, 0/62 patients respectively.

With the advances in molecular technology, the potential difficulties with culture have been circumvented by the use of the polymerase chain reaction ***Dowsett et al 2003.***[27]

PCR

PCR assays can detect the target DNA regardless of the viability of the bacteria ***Mapstone NP et al 1993***[66].

Yang H.T. 1993[113],***Mapstone NP et al 1993***[66],***Li.C. et al 1995***[57] demonstrated that nested PCR has a high sensitivity and specificity than one step PCR for samples with abundant bacterial flora

Asikainen et al 1994[4]***, Banatvala et al 1994***[8] reported that different sets of primers are used based on the urease gene, 16S ribosomal RNA, genes encoding H.pylori species-specific proteins or randomly selected DNA fragments.

Asikainen et al 1994 [4] showed prevalence of 0% in dental plaque by PCR from a total of 336 samples, and ***Riggio and Lennon 1999***[98] 38% of 29 samples in randomly selected patients, while ***Banatvala et al 1993***[7]

showed a prevalence of 86% of 21 samples in symptomatic H.pylori positive patients.

ELISA

Jones D.M., et al 1984[49]***, Marshall B.J 1984*** [70] reported that the antibody response to H.pylori infection was used for the diagnosis of this infection immediately following the discovery of H.pylori.

Mitchell et al 1987[80] stated that H.pylori infection is a chronic condition and IgG (subclasses 1 and 4) is the predominant immunoglobulin class even in children. IgG are present at the mucosal level and detected in virtually all blood samples.

Kupier.S.E. et al 1993[54] reported that H.pylori almost constantly induces a specific systemic immune response which may reflect the antibodies produced at the gastric mucosal level, while only 2% of patients fail to seroconvert.

Everhart JE et al 2000[32] found that the serological testing for H.pylori antibodies using an ELISA has a reported sensitivity and specificity of 91% and 97%.

RELATIONSHIP BETWEEN POOR ORAL HYGIENE, PERIODONTAL DISEASE ATTRIBUTES AND HELICOBACTER PYLORI INFECTION

Nabwera HM, Logan RP 1999[84] recently suggested that the primary extragastric reservoir for H.pylori is the oral cavity.

Since human infection by this pathogen appears to be involving an oral route, it seems biologically plausible that oral health status directly or indirectly influences the process of H.pylori infection or reinfection.

Bielanski 1999[10] reported positive association between poor oral health and H.pylori. He performed a large epidemiological investigation using urea – breath test in over 10,000 subjects, which revealed that periodontitis significantly increases the risk of gastric H.pylori infection.

Riggio and Lennon 1999[98] reported in their study, that when subgingival plaque samples were obtained from periodontal pockets at least 5mm deep, 33% of the pocket sites analyzed were H.pylori positive and 38% of the 29 subjects with moderate to severe periodontal pocketing were positive for H.pylori.

Avcu .N. et al 2001[5] correlated H.pylori positivity in dental plaque with OHI scores. The oral hygiene status was assessed by the Oral Hygiene Index of Greene and Vermillion. The positivity was 28.5%, 90.2% or 100% in patients with good, fair or poor OHI scores respectively.

Ozdemir A et al 2001[91] evaluated the oral hygiene status of gastritis patients using the Quigley Hein plaque index. The study did not report on the relationship of plaque scores with H.pylori infection.

Berroteran A. et al 2002[9] reported that there was no correlation between H.pylori infection and dental hygiene as assessed by the Plaque Index of Silness and Loe. He also reported no correlation between periodontal disease and H.pylori infection.

Dye.B A. et al 2002[29] reported from their study based on the data from the first phase of the Third National Health and Nutrition Examination survey, that periodontal disease characterized by deep periodontal pockets, may be associated with H.pylori infection in adults in the United States. They reported that periodontal pockets with a depth ≥ 5 mm were associated with increased odds of H pylori seropositivity after adjustment of sociodemographic factors.

Choudary CR et al 2003[19] suggested that periodontal health would determine whether oral H.pylori may or may not cause gastric infection.

Pradeep S. Anand et al 2006[94] examined the oral hygiene status of patients using the Oral Hygiene Index of Greene and Vermillion. The oral hygiene status of the patients was classified into good, fair and poor depending on their oral hygiene scores. Among cases 34 had poor oral hygiene, 25 had fair and 6 had good oral hygiene. Among controls 28 had poor, 33 had fair and eight had good oral hygiene. The study reported little correlation between oral hygiene status and H.pylori infection.

In the above study the periodontal status of the patients was also examined as a dichotomous variable and the patients described as either healthy or diseased. Among cases 30 of 65 subjects had periodontal disease compared to only 20 of 69 subjects among controls. Although there were more periodontal disease subjects among cases than controls, the observed difference was not statistically significant and they concluded that there is no association between periodontal disease attributes and H. pylori infection.

MATERIALS AND METHODS

STUDY DESIGN AND SUBJECT SELECTION

On approval from the Institutional ethical committee, the study was conducted at the Department of Medical Gastroenterology, Govt. General Hospital, Chennai from November 2008 – June 2009. The study included 110 patients with various symptoms relating to the gastrointestinal tract most commonly epigastric pain and dyspepsia and clinical indications for an upper gastrointestinal endoscopy. The patients were of either sex and in the age range from 17 to 76 years.

A written informed consent was obtained from all patients. The details of complete history and clinical features of the subjects undergoing endoscopy were obtained.

Preprocedure preparations for oesophagogastroendoscopy were performed according to standard methods. Prior to endoscopy assessment of oral hygiene status, clinical parameters-probing depth and clinical attachment level were measured and plaque samples were obtained for identification of H.pylori by rapid urease test and culture.

Biopsies of gastric tissue were collected from the antrum of patients undergoing endoscopy and specimens were used for 1) histopathology study 2) culture 3) rapid urease test 4) Gram and Giemsa staining for identification of H.pylori infection.

Venous blood samples were collected from the patients for serological diagnosis of H.pylori infection.

INCLUSION CRITERIA

All patients with complaints suggestive of upper gastrointestinal disease –i.e.; dyspepsia and who were to undergo endoscopy for the same were included in the study.

EXCLUSION CRITERIA

1. Patients with active bleeding ulcers
2. Post gastrectomy individuals
3. Patients with history of proton pump inhibitor within 2 weeks of endoscopy or antibiotic intake within one month prior to the study.
4. Patients with history of chronic use of NSAIDS.
5. Patients who have undergone oral prophylaxis within past 6 months.
6. Patients on mouth rinses.

The subjects were categorized into two groups cases (n = 55) and controls (n = 55).

Subjects with clinical symptoms and a positive test for any of the three diagnostic tests (histopathology, or rapid urease test on antral biopsy specimens or serology) were cases.

Subjects with clinical symptoms and a negative test for H.pylori serology or negative rapid urease test and histopathology of antral biopsy specimen were controls.

STUDY PROTOCOL

1) Institutional Ethical committee approval

2) Medical history and informed consent
3) Assessment of oral hygiene status by Simplified Oral Hygiene Index –Greene and Vermilion 1964.[42]
4) Periodontal examination using clinical parameters – probing depth and clinical attachment level.
5) Collection of plaque samples.
6) Collection of antral biopsy samples.
7) Collection of blood samples
8) Rapid urease test
9) Culture.
10) Histopathology
11) ELISA for detection of IgG antibody to H.pylori.
12) Statistical analysis

A detailed history and informed consent were obtained from all the subjects after explaining the study procedure.

The study variables obtained from the patient's history were age, gender, socioeconomic status, handling of animals, smoking(ex-smoker, current smoker, non-smoker) and alcohol consumption(current, past, never).

Socioeconomic status of the patient was classified as Upper, Upper middle, Lower middle; Upper lower and Lower based on Kuppuswamy's Socioeconomic Status Scale 2007.The original total scores for each of these groups is as given in the table.

KUPPUSWAMY'S SOCIOECONOMIC STATUS SCALE 2007[55]

(A) Education	**Score**
1. Profession or Honors	7
2. Graduate or post graduate	6
3. Intermediate or post high school diploma	5
4. High school certificate	4
5. Middle school certificate	3
6. Primary school certificate	2
7. Illiterate	1

(B) Occupation	**Score**
1. Profession	10
2. Semi-Profession	6
3. Clerical, Shop-owner, Farmer	5
4. Skilled worker	4
5. Semi-skilled worker	3
6. Unskilled worker	2
7. Unemployed	1

(C) Family income per Month (in Rs) - original	Score	Modified for 2007
1. =2000	12	=19575
2. 1000-1999	10	9788-19574
3. 750-999	6	7323- 9787
4. 500-749	4	4894- 7322
5. 300-499	3	2936-4893
6. 101-299	2	980-2935
7. =100	1	=979

TOTAL SCORE	SOCIOECONOMIC CLASS
26-29	Upper (I)
16-25	Upper Middle(II)
11-15 Middle	Lower middle (III)
5-10 Lower	Upper lower (IV)
<5	Lower(V)

In the present study the SES was classified as Upper, Middle and Lower because of small sample size. The total scores for these three groups that were used to arrive at the socioeconomic status of the patients were as follows: 26-29 Upper; 11-25 Middle; <5-10 Lower.

The oral hygiene status was assessed using the Simplified Oral Hygiene Index (OHI-S) Greene and Vermilion – 1964, [42] using mouth mirror and explorer.

SIMPLIFIED ORAL HYGIENE INDEX (OHI – S) GREENE AND VERMILION 1964[42]

Teeth examined – 16, 11, 26, 46, 31, and 36. If any of these teeth is missing, immediate distal tooth is examined.

Surfaces examined – buccal surfaces of upper molars, lingual surfaces of lower molars and labial surface of upper and lower incisors.

The OHI – S consists of 2 components.

A. Simplified Debris Index (DI – S)

B. Simplified Calculus Index (CI – S)

DEBRIS INDEX – SIMPLIFIED

SCORING CRITERIA

0 - No debris or stain present

1 - Soft debris covering < $1/3^{rd}$ of the tooth surface or the presence of extrinsic stains without debris, regardless of the surface area covered

2 - Soft debris covering > $1/3^{rd}$ but < $2/3^{rd}$ of the exposed tooth surface.

3 - Soft debris covering > $2/3^{rd}$ of the exposed tooth surface.

$$\text{Debris index simplified score per person} = \frac{\text{Sum of debris score per tooth surface}}{\text{Number of surfaces examined}}$$

CALCULUS INDEX – SIMPLIFIED

SCORING CRITERIA

0 - No calculus present

1 - Supragingival calculus covering < $1/3^{rd}$ of the exposed tooth surface.

2 - Supragingival calculus covering > $1/3^{rd}$ but < $2/3^{rds}$ of the exposed tooth surface (or)

The presence of individual flecks of sub gingival calculus around the cervical portion of the tooth (or) both.

3 - Supragingival calculus covering > $2/3^{rds}$ of the exposed tooth surface (or)

A continuous heavy band of sub gingival calculus around the cervical portion of the tooth (or) both.

$$\text{Calculus index – simplified score per person} = \frac{\text{Sum of calculus score per tooth surface}}{\text{Number of teeth examined}}$$

OHI – S score per person = DI - S + CI – S Score

Interpretation

Good = 0.0 – 1.2

Fair = 1.3 – 3.0

Poor = 3.1 – 6.0

Periodontal evaluation was done by measuring probing depth (PPD) and clinical attachment level (CAL) using mouth mirror and Williams's periodontal probe. Based on AAP classification 1999,[2] patients with periodontal disease – chronic periodontitis – localized (L) or generalized forms(G) were identified.

Localized - < 30% of sites involved and CAL more than1mm.

Generalized - > 30% of sites involved and CAL more than 1mm.

Subjects who were clinically healthy, with probing depth ≤ 3 mm and no clinical attachment loss were considered as healthy(H) subjects.

Probing depth (Grant 1965)[41]

Probing depth is measured from the gingival margin to the base of the pocket using a William's periodontal probe. The probe is passed under the gingiva along the circumference of the tooth. The probe inserted is always maintained parallel to the long axis of the tooth. Three measurements are made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth.

Clinical attachment level (Carranza) [86]

Clinical attachment level is measured from the cementoenamel junction to the base of the pocket using a William's periodontal probe.

When the gingival margin is located on the anatomic crown the level of attachment is determined by subtracting from the pocket depth, the distance from gingival margin to the cemento enamel junction. If both

are the same, the loss of attachment is zero. When the gingival margin coincides with the CEJ, the loss of attachment equals the pocket depth.

When the gingival margin is located apical to CEJ, the loss of attachment is greater than the pocket depth and therefore the distance between CEJ and gingival margin should be added to the pocket depth.

SPECIMEN COLLECTION

Patients were instructed to fast overnight before endoscopy.

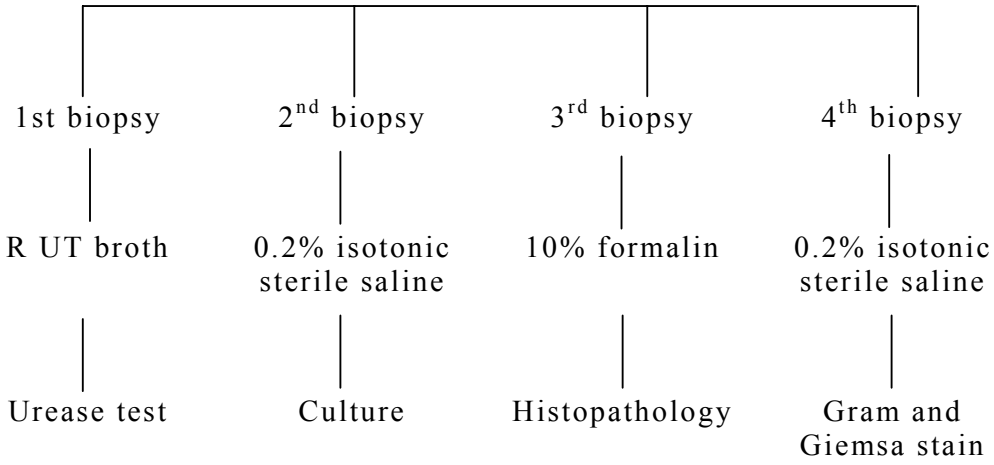
Prior to endoscopic examination of the patients, plaque samples were collected. Dental plaque was removed from the tooth surfaces with a sterile periodontal curette. The sample was dispersed immediately into a vial containing 1ml of urea broth with phenyl red indicator to detect urease activity, and in another vial containing 0.2% sterile isotonic saline for culture.

Endoscopy was carried out with an Olympus fiber optic endoscope by a Gastroenterologist. Prior to specimen collection, the endoscope with biopsy forceps was rinsed thoroughly in water and soaked in 2% glutaraldehyde (CIDEX) for 20 minutes. The endoscope was thoroughly rinsed with sterile normal saline just before use. From each subject, four biopsy specimens were taken from antral mucosa 2 cm from the pylorus[35]. The specimens were used for rapid urease test, culture, histopathology, Gram and Giemsa staining.

The specimens for rapid urease test were inoculated into vials containing urea broth immediately and the specimens for culture were inoculated into vials containing 0.2% sterile isotonic saline. The

specimens for histopathological examination were placed in 10% formalin. The specimens for culture were transported in an ice box to the laboratory and plated onto culture media within one hour of obtaining the specimen.

BIOPSY



BLOOD

With aseptic precautions, 2.5ml of venous blood was collected from each patient, the serum separated and stored at -20^{o}C, till it was used for IgG antibody estimation.

PROCEDURE

RAPID UREASE TEST

To adapt to its special ecological niche, where the concentration of urea diffusing from blood to the gastric mucosa is low, H.pylori produces large amounts of urease. H.pylori urease also has the highest specific activity (36 ± 28 μmol/min mg of protein among bacterial ureases ***Mobley 1988.***[81] The other urease positive bacteria present in the gastric mucosa i.e., streptococci and staphylococci produce a lower

amount of urease which does not interfere in a short – time detection (<2h), rendering the method specific to H. pylori.[35]

In the oral cavity, urease positive microorganisms such as Streptococcus vestibularis and Actinomyces viscosus usually cannot give positive results within an hour. Hence rapid urease test has been used by several studies to detect the presence of H.pylori in dental plaque.

When a biopsy specimen or a plaque sample containing H.pylori is introduced into urea rich medium, the urease breaks the urea down into carbon dioxide and ammonia. The ammonium ion increases the pH, and a pH indicator eg.phenol red changes color in this case from yellow to red or violet.

UREA BROTH:

Stock solution A [1% phenol red solution (free acid) indicator]

1 gm of phenol red was dissolved in 32.5ml of 0.1 mol/l sodium hydroxide and made upto 100 ml with distilled water. The solution was autoclaved for 15 minutes at 121^{o}C.

Stock solution B (10% urea solution)

10 gm of urea was dissolved in 100ml of sterile distilled water under sterile precautions. The pH of the solution was adjusted to 6.8 and dispensed in 0.5 ml aliquots in sterile vials.

Two drops of 1% phenol red (above) was added to each vial containing 0.5ml of 10% urea solution.

The specimens for the rapid urease test – antral biopsy specimen and the plaque sample were inoculated into two separate vials containing the urea broth. They were observed for color change from yellow to pink at room temperature every minute for the first 10 minutes, every 10 minutes for one hour and thereafter hourly for 8 hours and then at 24 hours. The change occurring within 2 hours denotes a positive reaction.[35]

CULTURE[60]

The antral biopsy specimens and the plaque samples, were transferred to the microbiology lab within 20 minutes at 2-8^{o}c in an ice pack. The biopsy specimen was finely ground between two sterile frosted glass slides. Both the biopsy and the plaque samples were then inoculated onto selective – Campylobacter agar base – Skirrow's medium and non selective media – chocolate agar.

NON SELECTIVE MEDIA

Chocolate Agar

Peptone	:	1.0 gm
Meat extract	:	1.0gm
Sodium chloride	:	0.5gm
Agar agar	:	2 gm
Distilled water	:	100 ml
Defibrinated sheep blood	:	10%

SELECTIVE MEDIA

Skirrow's Campylobacter medium

i) Campylobacter agar base	:	39.5 gm
ii) Distilled water	:	1 liter
iii) Skirrow's supplement	:	1 vial consisting of
Vancomycin	:	10 gm
Polymyxin B	:	2500 IU
Trimethoprim	:	5 ml
iv Defibrinated sheep blood	:	50 ml

The plates were incubated immediately at 37^{o}C in a microaerophilic atmosphere in a candle jar or in a BOD (biological oxygen demand) incubator with Co_2 supply for 5 days.

Colony Characteristics

Chocolate agar plate in - BOD incubator	Growth obtained from 2 antral biopsies

The colonies showed the following morphology – circular, convex, translucent and glistening with slight hemolysis in blood agar.

The suspected colonies were confirmed by

1) ***Gram stain*** – gram negative curved bacilli were seen – spiral forms were less obvious.
2) ***Oxidase test*** – The suspected colony was streaked on the surface of an oxidase strip. An intense purple color within 5 seconds was noted as positive.

3) ***Catalase test*** – The suspected colony was introduced into hydrogen peroxide. Immediate production of gas bubbles was noted as positive.

4) ***Urease test*** – The suspected colony was introduced into 0.5ml of the urea broth. An instant color change from yellow to pink, which deepened in intensity, was noted as positive.

HISTOPATHOLOGY

The biopsy samples from the antrum were processed and stained with haematoxylin and eosin stain and examined for Helicobacter pylori.

CRUSH CYTOLOGY[97]

The specimen in the transport medium was crushed between two sterile glass slides.

Gram stain

One slide was air dried and heat fixed. The slide was first covered with methyl violet for one minute. The excess stain was poured off, gram's iodine added and after 1 minute washed and decolorized with acetone for 2-3 seconds. The acetone was washed off with water and counter stained with dilute carbol fuschin for one minute. The slide was then washed with water, blotted dry and observed under oil immersion objective. The smear was examined for the presence of gram negative helical bacteria.

Giemsa stain

The other slide was air dried and fixed with methanol for 3 minutes. 2 – 3 drops of undiluted Giemsa stain (Qualigenas, Glaxo) was added and kept for 5 minutes. The smear was then washed with water, blotted dry and seen under oil immersion objective. The organism appeared deep purple with the typical gull-wing morphology.

SEROLOGY

The serological detection of IgG antibodies to cellular components of H.pylori was done, using the **EUROIMMUNO ELISA Kit**.

Antigen: The antigen source is provided by the strain Lior 1, (Brussels) "of Helicobacter pylori. The cultured bacteria have been disrupted in alkaline buffer." The used antigen mixture contains all significant proteins as verified by SDS (sodium dodecyl sulphate) polyacrylamide gel electrophoresis.

Contents of the test kit:

1. Micro plate wells coated with antigens
2. Calibrator 1

   200 RU/ml (IgG, Human), ready for use
3. Calibrator 2

   20 RU/ml (IgG, Human), ready for use
4. Calibrator 3

   2 RU/ml (IgG, Human), ready for use
5. Positive control

   (IgG, Human), ready for use
6. Negative Control

   (IgG, Human), ready for use
7. Enzyme conjugate

   Peroxidase – Labeled anti – human IgG (rabbit), ready for use.
8. Sample buffer ready for use
9. Wash buffer

   10 xs concentrate
10. Chromogen / substrate solution

TMB / H_2O_2 ready for use

11. Stop solution

    0.5 sulphuric acid, ready for use

12. Test instruction

13. Protocol with target values

Serology was done for 110 patients. 3 calibrated standards provided by the manufacturer and serum samples of all the cases and controls were included.

Method:

- Serum samples were diluted 1: 101 before assay (10 µl of serum was diluted with 1ml of sample diluents).
- 100 µl of each calibrated standard or diluted sample was dispensed into the wells.
- The plate was incubated for 30 minutes at room temperature.
- The wells were washed thoroughly thrice using wash buffer, the micro plate was blotted on absorbent paper.
- 100 µl conjugate consisting of rabbit antihuman IgG conjugated with Horse radish peroxidase was added immediately into each well.
- The plate was incubated for 30 minutes at room temperature.
- Following incubation, the plate was washed 3 times with wash buffer.
- 100µl of chromogen / substrate solution was added into each well. The plate was incubated for 15 minutes. Green color developed in the wells.
- 100µl of stop solution (0.05m sulphuric acid) was added to each well in the same as the chromogen substrate to allow equal reaction times.
- Green color change to yellow on addition of the stop solution.

- The optical density was read at 450 nm in a micro plate reader within 30 minutes of adding the stop solution.

CALCULATION OF RESULTS

The optical density of each calibrator was plotted against its concentration and a curve was drawn through the points. The unknowns were read off the curve.

The kit recommends interpreting results as follows:

< 16 RU/ml –Negative

≥ 16 to <22 RU/ml – Borderline

≥ 22 RU/ml – Positive

ARMAMENTARIUM

Periodontal examination

Mouth mirror

William's periodontal probe

Explorer

Tweezers

Mask

Head cap

Gloves

Periodontal curettes

Sterile aliquots containing saline

Rapid Urease Test

Test tube

Beaker

Conical flask

Dropper

Forceps

Sterile aliquots

Culture

Culture (media plates)

Bacteriological loop

Sterile stick

Petri disk

Test tubes

Sterile aliquots

Frosted glass slides

Bunsen burner

Carbon dioxide incubator

Candle jar

Ice base

Histopathology

Plane glass slide

Microscope

ELISA

Test tubes

Wooden Rack

Measuring Jar

Bowls

Autoclaved plastic tips

ELISA washer

ELISA reader

Photograph No.1: Good oral hygiene in H.pylori positive patient

Photograph No.2: Fair oral hygiene in H.pylori positive patient

Photograph No.3: Poor oral hygiene in H.pylori positive patient

Photograph No.4: Periodontitis in H.pylori positive patient

Photograph No.5: Armamentarium for dental plaque and blood sampling

Photograph No.6: Pocket depth > 5mm in H.pylori positive patient

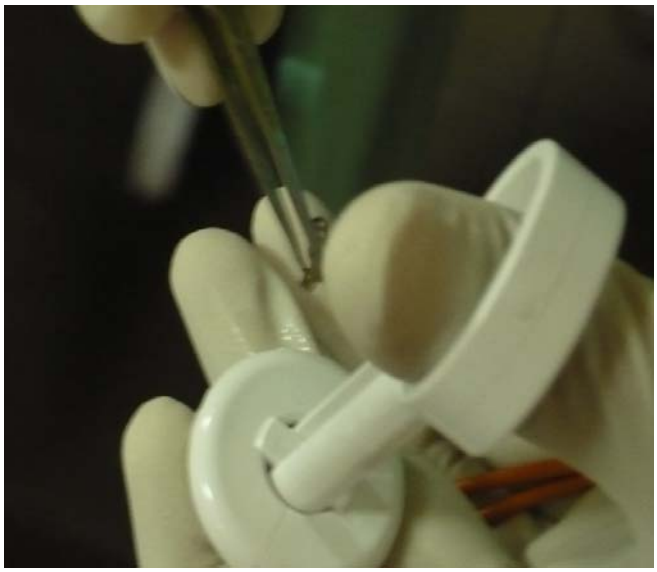
Photograph No.7: Aliquots with urea broth and saline for RUT and sample collection

Photograph No.8: Transport for samples

Photograph No.9: Collection of dental plaque sample

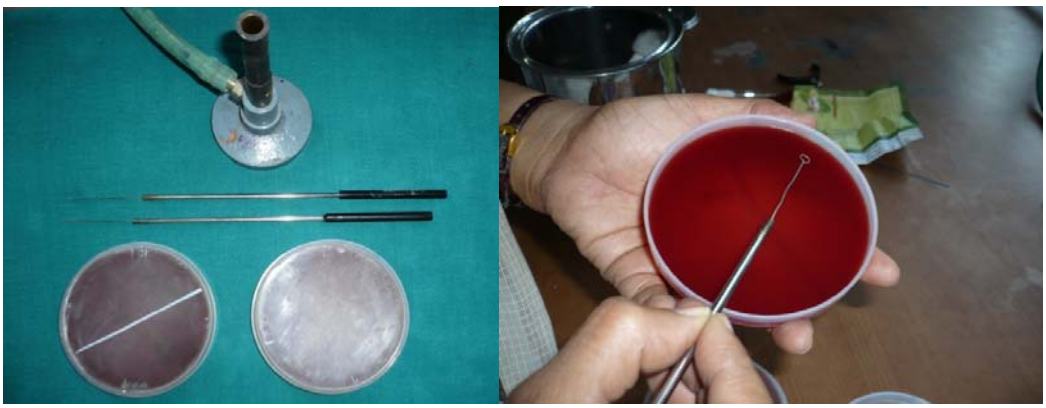
Photograph No. 10: Endoscope and biopsy forceps for antral biopsy

Photograph No. 11: Antral biopsy obtained during endoscopy by Gastroenterologist



Photograph No.12: Antral biopsy taken from the biopsy forceps

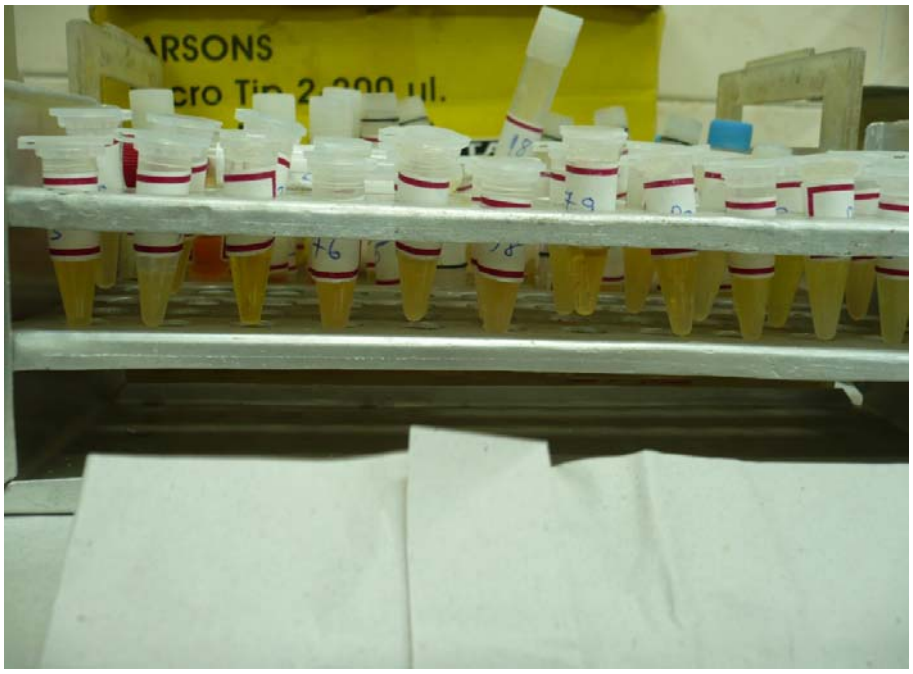
Photograph No.13: Collection of blood sample for ELISA



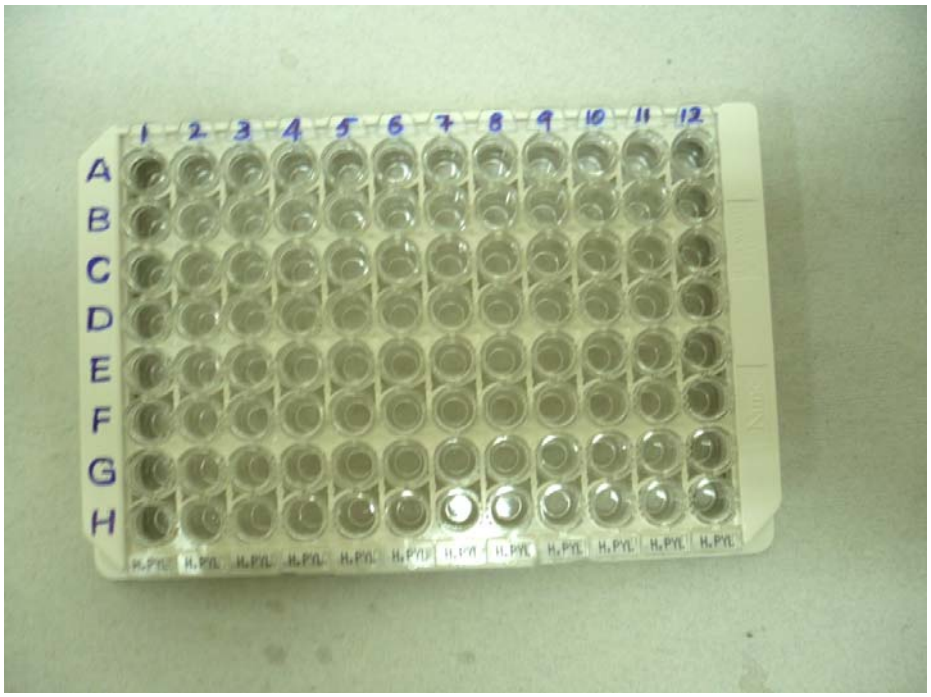
Photograph No.14: Armamentarium for culture (left), streaking of sample onto culture plate (right)

Photograph No. 15: Electron Microscope

Photograph No. 16: ELISA kit for Anti-H.pylori (IgG)



Photograph No. 17: Serum samples for ELISA



Photograph No. 18: Microplate wells coated with antigen

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS version 12.

Mean and Standard Deviation were estimated for different variables in each study group.

Normality of the data was tested in each group by using Kolmogrove Smirnov test.

Mean values were compared between two study groups by using either ***Student's Independent t-test*** or ***Mann-Whitney U-Test***.

Pearson's chi-square test was used to compare the proportions two study groups.

In the present study, p<0.05 was considered as the level of significance.

Statistical Formulae Used For Data Analysis

Pearson's Chi-square Test

The formula used was

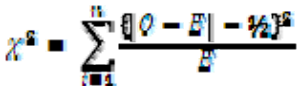
$$\chi^2 = \sum_{i=1}^{n} \left[\frac{(O - E)^2}{E} \right]$$

Where O = Observed frequency in a cell

E = Expected frequency in a cell

Pearson's Chi-square Test with Yates' Continuity Correction

The formula used was

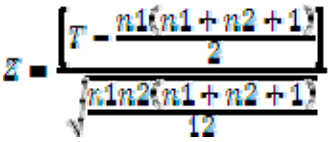


Where O = Observed frequency in a cell

E = Expected frequency in a cell

Mann-Whitney U-Test

The formula is



Where n1 and n2 are the sample sizes in Group I and Group II respectively.

T is the sum of the ranks for the n1 observations.

Student's Independent t-test

The formula used is

$$t = (\overline{X_1} - \overline{X_2}) \Big/ \sqrt{S_p^2/n_1 - S_p^2/n_2}$$

where $S_p^2 = [(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2] \Big/ (n_1 + n_2 - 2)$

$\overline{X_1}$ and $\overline{X_2}$ are the sample means;

RESULTS

One hundred and ten subjects were included in the study. The subjects were categorized into two groups – cases (n = 55) and controls (n = 55).

Table I & II shows the master chart of cases and controls with the variables, clinical parameters and tests done in both groups in the study.

Table III & Fig I shows the comparison of gender between cases and controls. Distribution of gender in both groups was similar.

Table IV & Fig II shows the comparison of handling of animals between cases and controls. 20 of 55 cases (36.4%) had contact with animals compared to only 4 of 55 controls (7.3%). This difference was found to be significant with a P value of 0.001.

Table V & Fig III shows the comparison of socioeconomic status between cases and controls. There were more patients of lower socioeconomic status in both cases (n=42/55), and controls (n=41/55) but it was not found to be statistically significant (P= 1.00).

Table VI shows the comparison of mean values between cases and controls. The mean age in controls (48$\pm$12.5) was higher than cases (44.4$\pm$15.2). However there was no significant difference in mean age between cases and controls. The mean socio-economic status score in controls (10.0 ± 4.2) was higher than in cases (9.4 ± 4.1). However there was no significant difference in mean SES score between cases and controls (P = 0.27). The mean ELISA (RU/ml) score in cases (159±32) was significantly higher than in controls (54±36) P (< 0.0001). The mean OHI score in cases (2.05 ± 1.16) was higher than in controls

(1.98 ± 1.07). However, there was no significant difference in OHI scores between cases and controls (P=0.75).

Table VII shows the comparison of smoking habit and alcohol consumption between cases and controls. The proportion of current smokers in cases (38.2%) was slightly higher than controls (29.1). However there was no significant difference in the proportion of current smokers between the two study groups (P = 0.59). The proportion of people currently having the habit of alcohol consumption in cases (41.8%) was slightly lower than controls (43.6%). However there was no significant difference between cases and controls (P = 0.67).

Table VIII & Fig IV shows the comparison of OHI score between cases and controls. Among cases 19 subjects (34.5%) had good oral hygiene, 25(45.5%) had fair and 11 (20.0%) had poor oral hygiene. Among controls 17(30.9%), 28 (50.9%), and 10 (18.2%) had good, fair and poor oral hygiene respectively. The observed difference in the oral hygiene status between the two groups was not found to be statistically significant (P = 0.85).

Table IX & Fig V shows the comparison of periodontal disease and pocket depth > 5mm between cases and controls. 27 (49.1%) subjects among cases had periodontal disease with pocket depth of ≥ 5mm in at least one site compared to only 8 (14.5%) subjects among controls. This difference was found to be statistically significant (P < 0.001).

Table X & Fig VI shows the comparison of RUT (P) and culture (P) between cases and controls. 48 cases (87.3%) had a positive rapid urease test compared to 29 (52.7%) subjects among controls. This difference was found to be statistically significant with P value of <

0.001. H.pylori could not be cultured from dental plaque in any of the 55 cases or controls.

Table XI shows the comparison of RUT (B), culture (B), Gram and Giemsa stain between cases and controls. On antral biopsy specimens, all 55 cases (100%) had positive rapid urease test, while 55 controls had negative RUT. 2 cases tested positive on culture, 40 cases (72.7%) positive on Gram staining and 50 cases (90.9%) positive on Giemsa staining for H.pylori.

Results of histopathological examination of antral biopsy samples revealed 40 cases with chronic active gastritis, 4 cases with features suggestive of malignancy (adenocarcinoma), 2– cases with acute gastritis, 5 cases of atrophic gastritis and 4 cases of intestinal metaplasia. Among controls the picture was that of chronic nonactive gastritis or lymphocytic infiltration

Table XII shows the results of multiple logistic regression analysis. The variables found to be statistically significant in the university analysis-contact with animals; periodontal disease status with pocket depth of ≥ 5 mm and RUT (P) positive were included in the multivariate logistic regression analysis. In the multivariate analysis, it was found that the observed difference in the above three variables between the two groups were statistically significant.

Tables XIII and IV shows the association of OHI and Periodontal disease with RUT (P) between cases and controls. The results showed that the association of OHI and Periodontal disease with RUT(P) in cases was not significant, but among controls the association of periodontal disease with RUT(P) was significant(P=0.005).

TABLE –I MASTER CHART-CASES

Code No	Age	Sex	Smoker			Alcohol			Contact with Animals	SES Score	SES Status	OHI Score	OHI Status	Periodontal disease status	Pocket depth > 5mm / site	RUT		Culture		ELISA (RU/ml)	Gram Stain	Giemsa stain
			Current	Ex	No	Current	Past	No								P	B	P	B			
001	35	M	✓			✓			No	7	(U) Lower	2.0	Fair	H	-	+	+	-	-	190	+	+
002	67	M	✓			✓			Yes Cats	6	(U) Lower	2.9	Fair	G	+	+	+	-	-	136	+	+
003	75	M		✓			✓		No	16	(U) Middle	3.2	Poor	G	+	+	+	-	-	140	+	+
004	45	M	✓			✓			No	11	(L) Middle	3.2	Poor	G	+	-	+	-	-	168	+	+
005	21	M			✓			✓	Yes Dogs Goats	8	(U) Lower	1.3	Good	H	-	+	+	-	-	> 200	+	+
006	67	M		✓			✓		Yes Cows Dogs	12	(L) Middle	1.0	Good	H	-	+	+	-	-	114	-	+
007	66	M			✓	✓		✓	No	11	(L) Middle	2.2	Fair	H	-	+	+	-	-	136	+	+
008	39	M		✓		✓			No	7	(U) Lower	1.3	Good	H	-	+	+	-	-	138	+	+
009	35	M		✓		✓			No	6	(U) Lower	2.1	Fair	H	-	+	+	-	-	135	+	+
010	45	M	✓			✓			No	7	(U) Lower	2.9	Fair	H	-	+	+	-	-	> 200	+	+
011	30	M	✓			✓			Yes Cats	16	(U) Middle	3.6	Poor	G	+	+	+	-	-	> 200	+	+
012	51	M		✓		✓			No	10	(U) Lower	2.0	Fair	L	+	+	+	-	-	114	+	+
013	52	M	✓			✓			No	8	(U) Lower	3.3	Poor	G	+	+	+	-	-	188	+	+
014	33	F			✓			✓	Yes Cats	9	(U) Lower	1.2	Good	H	-	+	+	-	-	> 200	+	+
015	70	M		✓			✓		Yes Goats	19	(U) Middle	2.1	Fair	L	+	+	+	-	-	> 200	+	+
016	30	M			✓			✓	No	16	(U) Middle	0.6	Good	H	-	+	+	-	-	> 200	+	+
017	45	F			✓			✓	No	9	(U) Lower	2.7	Fair	H	-	+	+	-	-	164	-	+
018	33	F			✓			✓	No	5	(U) Lower	0.8	Good	H	-	-	+	-	-	110	+	+
019	44	M		✓			✓		No	8	(U) Lower	2.6	Fair	G	+	+	+	-	-	198	-	+
020	40	M	✓					✓	Yes Goats	6	(U) Lower	3.4	Poor	G	+	+	+	-	-	90	+	+
021	69	M		✓			✓		No	14	(U) Middle	2.0	Fair	G	+	+	+	-	-	190	+	+
022	70	M			✓	✓			Yes Cats	5	Lower	2.0	Fair	G	+	-	+	-	-	170	-	+
023	47	M		✓		✓			Yes Goats	8	(U) Lower	2.2	Fair	L	+	-	+	-	-	96	+	+
024	18	F			✓			✓	Yes Dogs	4	Lower	0.46	Good	H	-	-	+	-	-	170	+	+
025	21	F							Yes Goats	10	(U) Lower	0.46	Good	H	-	-	-	-	-	> 200	+	+

Code No	Age	Sex	Smoker			Alcohol			Contact with Animals	SES Score	SES Status	OHI Score	OHI Status	Periodontal disease status	Pocket depth > 5mm / site	RUT		Culture		ELISA (RU/ml)	Gram Stain	Giemsa stain
			Current	Ex	No	Current	Past	No								P	B	P	B			
026	50	M	✓			✓			Yes Hens	9	(U) Lower	4.6	Poor	G	+	+	+	-	-	138	+	+
027	62	M	✓			✓			Yes Cows	10	(U) Lower	2.0	Fair	G	+	+	+	-	-	156	+	+
028	44	M	✓					✓	No	7	(U) Lower	3.0	Fair	H	-	+	+	-	-	130	+	+
029	33	F			✓			✓	Yes Dogs	3	Lower	0.6	Good	H	-	+	+	-	-	180	+	+
030	65	M	✓			✓			No	16	(U) Middle	4.0	Poor	G	+	+	+	-	-	136	+	+
031	57	M	✓			✓			Yes Cows	8	(U) Lower	3.0	Fair	L	+	+	+	-	-	164	+	+
032	46	M			✓	✓			Yes Goats	9	(U) Lower	1.8	Fair	L	+	+	+	-	-	198	+	+
033	47	M	✓			✓			Yes Cats	14	(L) Middle	2.7	Fair	G	+	+	+	-	-	150	+	+
034	65	M		✓			✓		No	24	(U) Middle	2.4	Fair	L	+	+	+	-	-	167	+	+
035	36	F			✓			✓	No	8	(U) Lower	0.7	Good	H	-	+	+	-	-	158	+	+
036	35	F			✓			✓	No	7	(U) Lower	1.7	Fair	H	-	+	+	-	-	118	-	+
037	19	M			✓			✓	No	5	(U) Lower	1.1	Good	H	-	+	+	-	-	174	+	+
038	55	M		✓				✓	No	10	(U) Lower	5.0	Poor	G	+	+	+	-	-	136	+	+
039	47	M	✓					✓	Yes Cows	8	(U) Lower	2.1	Fair	H	-	+	+	-	-	170	+	+
040	35	M			✓			✓	No	7	(U) Lower	0.6	Good	H	-	+	+	-	-	168	+	+
041	50	M	✓					✓	No	11	(L) Middle	1.8	Fair	G	+	+	+	-	-	170	+	+
042	38	M			✓	✓			No	9	(U) Lower	0.9	Good	H	-	+	+	-	-	168	+	+
043	35	F	✓					✓	No	8	(U) Lower	3.1	Poor	G	+	+	+	-	-	106	-	+
044	35	M	✓			✓			No	9	(U) Lower	3.2	Poor	L	+	+	+	-	-	150	+	+
045	30	M	✓			✓			No	10	(U) Lower	1.6	Fair	H	-	+	+	-	-	135	-	+
046	35	F			✓			✓	No	8	(U) Lower	1.2	Good	H	-	+	+	-	-	140	-	-
047	45	M	✓			✓			Yes Dogs Goats	10	(U) Lower	2.3	Fair	L	+	+	+	-	-	155	-	-
048	46	F			✓			✓	No	8	(U) Lower	1.2	Fair	L	+	+	+	-	-	160	-	+
049	67	M	✓			✓			Yes Dogs	19	(U) Middle	2.0	Fair	G	+	+	+	-	-	130	-	-
050	26	F			✓			✓	No	9	(U) Lower	1.0	Good	H	-	+	+	-	-	146	-	-
051	17	F			✓			✓	No	6	(U) Lower	0.6	Good	H	-	+	+	-	-	127	-	+
052	44	F			✓			✓	No	6	(U) Lower	0.8	Good	H	-	+	+	-	-	137	-	+
053	65	M		✓			✓		No	9	(U) Lower	5.0	Poor	G	+	+	+	-	-	200	+	-
054	35	F			✓			✓	No	4	(U) Lower	0.6	Good	H	-	+	+	-	-	125	-	+
055	33	M	✓			✓			No	9	(U) Lower	1.0	Good	H	-	-	+	-	--	136	-	+

H-Healthy; G- Generalized chronic periodontitis; L- Localized chronic periodontitis

Table –II MASTER CHART- CONTROLS

Code No	Age	Sex	Smoker			Alcohol			Contact with Animals	SES Score	SES Status	OHI Score	OHI Status	Periodontal disease status	Pocket depth > 5mm / site	RUT		Culture		ELISA (RU/ml)	Gram Stain	Giemsa stain
			Current	Ex	No	Current	Past	No								P	B	P	B			
001	46	M	✓					✓	No	12	(L) Middle	2.9	Fair	H	-	-	-	-	-	12	-	-
002	46	M	✓					✓	No	6	(U) Lower	4.3	Poor	H	-	-	-	-	-	14	-	-
003	65	M	✓			✓			No	8	(U) Lower	3.1	Poor	H	-	-	-	-	-	22	-	-
004	45	M		✓				✓	No	8	(U) Lower	1.1	Good	H	-	+	-	-	-	19	-	-
005	37	M	✓				✓		No	10	(U) Lower	0.3	Good	H	-	+	-	-	-	26	-	-
006	65	M			✓	✓			No	10	(U) Lower	2.5	Fair	G	+	+	-	-	-	32	-	-
007	47	F			✓			✓	No	4	(U) Lower	0.6	Good	H	-	+	-	-	-	12	-	-
008	40	F			✓			✓	No	9	(U) Lower	0.6	Good	H	-	-	-	-	-	70	-	-
009	43	M	✓			✓			No	9	(U) Lower	4.0	Poor	G	+	+	-	-	-	48	-	-
010	26	M			✓			✓	No	16	(U) Middle	0.9	Good	H	-	-	-	-	-	48	-	-
011	30	M			✓	✓			Yes Cows	10	(U) Lower	2.0	Fair	H	-	+	-	-	-		-	-
012	40	F							No	6	(U) Lower	3.6	Poor	G	+	+	-	-	-	78	-	-
013	57	M	✓			✓			No	18	(U) Middle	1.8	Fair	H	-	-	-	-	-	62	-	-
014	50	M	✓			✓			No	9	(U) Lower	2.0	Fair	H	-	+	-	-	-	70	-	-
015	58	M		✓				✓	Yes Cows	10	(U) Lower	2.4	Fair	H	-	+	-	-	-		-	-
016	70	M							No	8	(U) Lower	1.8	Fair	H	-	-	-	-	-	78	-	-
017	50	M	✓					✓	Yes Dogs, Cows, Hens	8	(U) Lower	2.4	Fair	G	+	+	-	-	-	78	-	-
018	40	F			✓			✓	No	14	(U) Middle	0.6	Good	H	-	-	-	-	-	60	-	-
019	36	M			✓	✓			No	8	(U) Lower	0.4	Good	H	-	+	-	-	-	94	-	-
020	47	M			✓	✓			No	16	(U) Middle	3.2	Poor	H	-	-	-	-	-	24	-	-
021	50	F			✓			✓	No	5	(U) Lower	0.9	Good	H	-	+	-	-	-	20	-	-
022	70	F			✓			✓	No	2	Lower	3.4	Poor	G	+	+	-	-	-	14	-	-
023	46	M			✓	✓			No	9	(U) Lower	2.6	Fair	H	-	+	-	-	-	92	-	-
024	46	M	✓			✓			No	18	(U) Middle	2.0	Fair	H	-	-	-	-	-	100	-	-
025	76	M		✓			✓		No	15	(L) Middle	2.8	Fair	H	-	-	-	-	-	18	-	-
026	55	M	✓	□		□	✓		No	8	(U) Lower	0.6	Good	H	-	+	-	-	-	74	-	-
027	35	M	✓			✓			No	15	(L) Middle	3.2	Poor	L	+	+	-	-	-	50	-	-
028	38	M	✓			✓			No	10	(L) Lower	2.2	Fair	H	-	-	-	-	-	104	-	-
029	70	M			✓	✓			No	9	(L) Lower	3.0	Fair	H	-	+	-	-	-	60	-	-
030	39	M		✓		✓			No	9	(L) Lower	1.6	Fair	H	-	-	-	-	-	18	-	-

H-Healthy; G- Generalized chronic periodontitis; L- Localized chronic periodontitis

Code No	Age	Sex	Smoker			Alcohol			Contact with Animals	SES Score	SES Status	OHI Score	OHI Status	Periodontal disease status	Pocket depth > 5mm / site	RUT		Culture		ELISA (RU/ml)	Gram Stain	Giemsa stain
			Current	Ex	No	Current	Past	No								P	B	P	B			
031	65	M		✓			✓		No	9	(U) Lower	2.3	Fair	H	-	+	-	-	-	78	-	-
032	46	M	✓			✓			No	18	(U) Middle	1.5	Fair	H	-	-	-	-	-	120	-	-
033	65	M	✓			✓			No	13	(U) Middle	2.2	Fair	H	-	-	-	-	-	72	-	-
034	30	M			✓	✓			No	9	(U) Lower	1.8	Fair	H	-	+	-	-	-	18	-	-
035	48	M	✓			✓			No	6	(U) Lower	1.2	Good	H	-	+	-	-	-	98	-	-
036	45	M			✓			✓	No	10	(U) Lower	1.5	Fair	H	-	-	-	-	-	3	-	-
037	40	M			✓	✓			No	8	(U) Lower	2.0	Fair	H	-	-	-	-	-	6	-	-
038	38	M		✓				✓	No	10	(U) Lower	4.0	Poor	H	-	+	-	-	-	8	-	-
039	25	M			✓			✓	No	25	(U) Middle	0.3	Good	H	-	-	-	-	-	18	-	-
040	59	M	✓			✓			No	15	(L) Middle	1.4	Fair	H	-	-	-	-	-	22	-	-
041	41	M							No	11	(L) Middle	0.6	Good	H	-	-	-	-	-	34	-	-
042	62	M		✓				✓	No	15	(L) Middle	2.2	Fair	H	-	+	-	-	-	64	-	-
043	68	M		✓		✓			No	8	(U) Lower	1.9	Fair	H	-	-	-	-	-	20	-	-
044	60	M		✓		✓			No	10	(U) Lower	2.8	Fair	H	-	+	-	-	-	58	-	-
045	42	M	✓			✓			Yes Cows	10	(U) Lower	2.0	Fair	L	+	+	-	-	-	96	-	-
046	43	F			✓			✓	No	5	(U) Lower	1.2	Good	H	-	-	-	-	-	23	-	-
047	43	F	✓			✓			No	10	(U) Lower	2.2	Fair	H	-	+	-	-	-	40	-	-
048	27	F			✓			✓	No	9	(U) Lower	0.6	Good	H	-	+	-	-	-	16	-	-
049	39	F			✓			✓	No	9	(U) Lower	3.1	Poor	H	-	-	-	-	-	26	-	-
050	41	F			✓			✓	No	9	(U) Lower	0.9	Good	H	-	+	-	-	-	18	-	-
051	45	F			✓			✓	No	4	(U) Lower	2.0	Fair	H	-	-	-	-	-	106	-	-
052	42	F			✓			✓	No	5	(U) Lower	0.4	Good	H	-	+	-	-	-	112	-	-
053	45	F			✓			✓	No	10	(U) Lower	0.9	Good	H	-	-	-	-	-	14	-	-
054	60	F			✓			✓	No	8	(U) Lower	2.6	Fair	H	-	-	-	-	-	126	-	-
055	67	F			✓			✓	No	4	(U) Lower	4.1	Poor	G	+	+	-	-	-	53	-	-

TABLE-III

COMPARISON OF GENDER BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
Gender	Male	16	29.1	16	29.1	
	Female	39	70.9	39	70.9	1.00(NS)

FIGURE I

DISTRIBUTION OF GENDER IN CASES AND CONTROLS

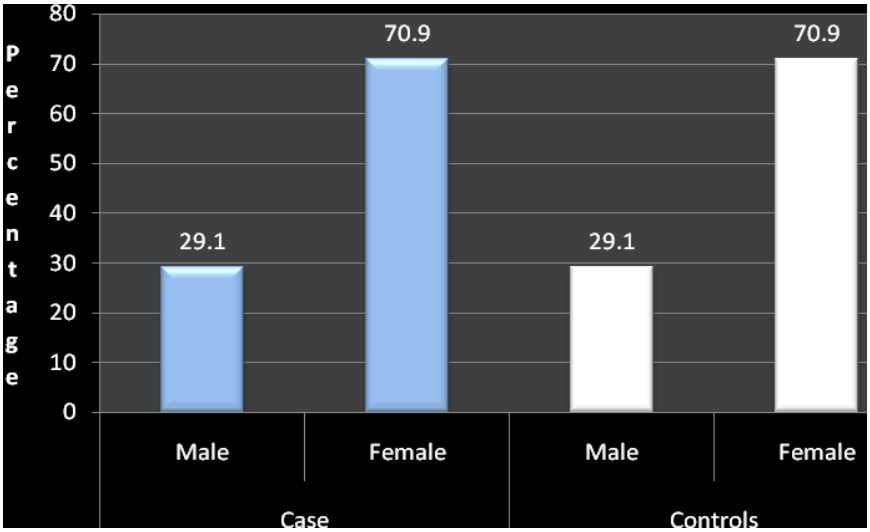


TABLE-IV

COMPARISON OF HANDLING OF ANIMALS BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
Contact with animals	No	35	63.6	51	92.7	0.001 (Sig.)
	Yes	20	36.4	4	7.3	
Type of animal	Cat	6	30.0	0	0.0	0.07 (N.S)
	Dog	3	15.0	0	0.0	
	Cow	3	15.0	3	75.0	
	Goat	3	15.0	0	0.0	
	Hen	1	5.0	0	0.0	
	Dog, Cow, Goat	0	0.0	1	25.0	
	Dog, Goat	3	15.0	0	0.0	
	Cow, Dog	1	5.0	0	0.0	

FIGURE II

COMPARISON OF HANDLING OF ANIMALS BETWEEN CASES AND CONTROLS

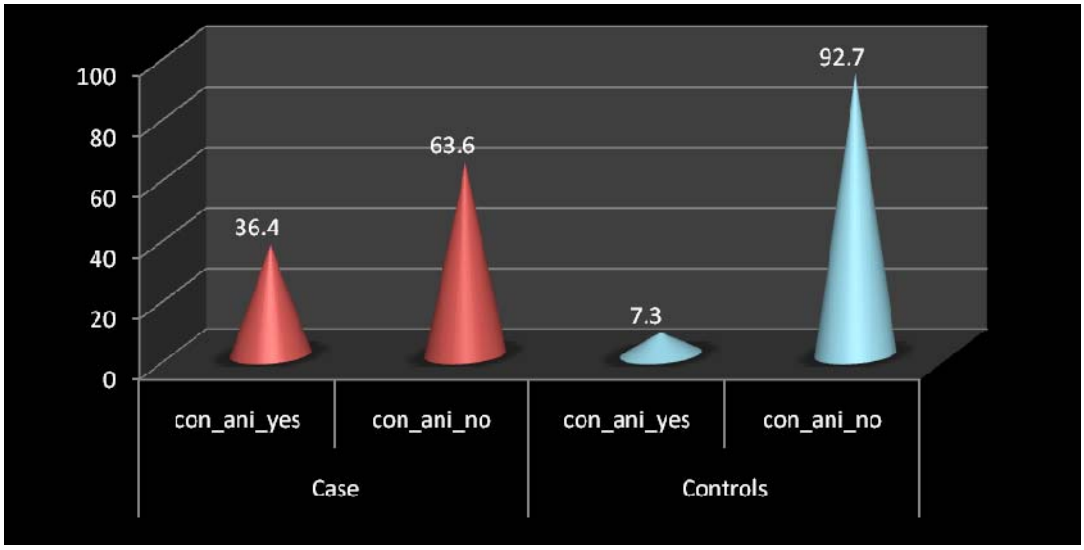


TABLE-V

COMPARISON OF SOCIOECONOMIC STATUS BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
SES	Lower	42	76.4	41	74.5	1.00 (N.S.)
	Middle	13	23.6	14	25.5	

FIGURE III

COMPARISON OF SOCIOECONOMIC STATUS BETWEEN CASES AND CONTROLS

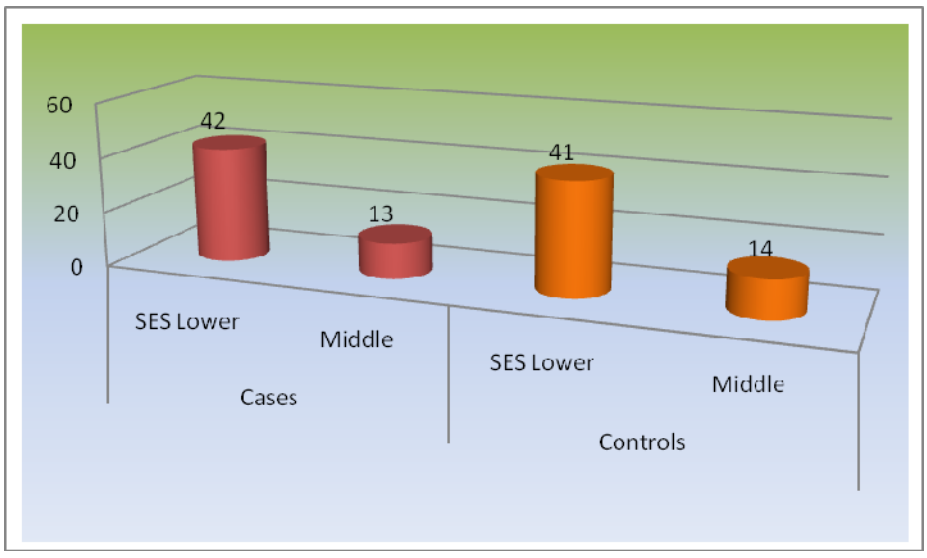


TABLE VI

COMPARISON OF MEAN VALUES BETWEEN CASES AND CONTROLS

Variable	Group	Mean ± S.D.	P-value*
Age	Cases	44.4 ± 15.2	0.17 (N.S.)
	Controls	48.2 ± 12.5	
OHI Score	Cases	2.05 ± 1.16	0.75 (N.S.)
	Controls	1.98 ± 1.07	
ELISA	Cases	159 ± 32	<0.0001 (Sig.)
	Controls	54 ± 36	
SES Score	Cases	9.4 ± 4.1	0.27** (N.S.)
	Controls	10.0 ± 4.2	

*Student's independent t-test was used to calculate the P-value.
****** Mann-Whitney U-test was used to calculate the P-value.

TABLE-VII

COMPARISON OF SMOKING HABIT AND ALCOHOL CONSUMPTION BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
Smoker	Non-smoker	22	40.0	26	47.3	0.59(NS)
	Current	21	38.2	16	29.1	
	Ex- smoker	12	21.8	13	23.6	
Alcohol	Not at all	24	43.6	26	47.3	0.67(NS)
	Current	23	41.8	24	43.6	
	Past	8	14.5	5	9.1	

TABLE-VIII

COMPARISON OF OHI SCORE BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
OHI-SCORE	Poor	11	20.0	10	18.2	0.85 (N.S.)
	Fair	25	45.5	28	50.9	
	Good	19	34.5	17	30.9	

FIGUREIV

COMPARISON OF OHI SCORE BETWEEN CASES AND CONTROLS

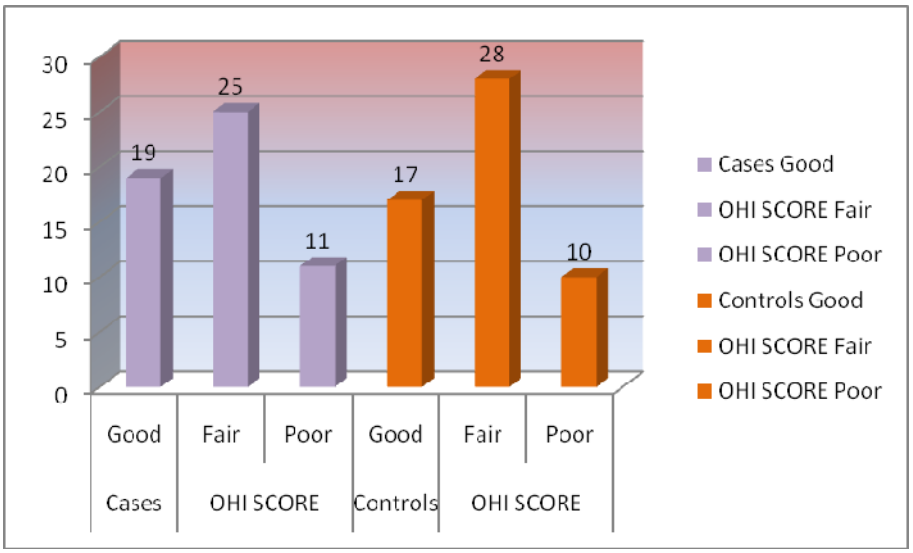


TABLE-IX

COMPARISON OF PERIODONTAL DISEASE AND POCKET DEPTH ≥ 5MM BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
Periodontal disease	Healthy	28	50.9	47	85.5	<0.0001 (Sig.)
	Periodontitis	27	49.1	8	14.5	
Pocket depth > 5mm / site	No	28	50.9	47	85.5	<0.0001 (Sig.)
	Yes	27	49.1	8	14.5	

FIGURE V

COMPARISON OF PERIODONTAL DISEASE BETWEEN CASES AND CONTROLS

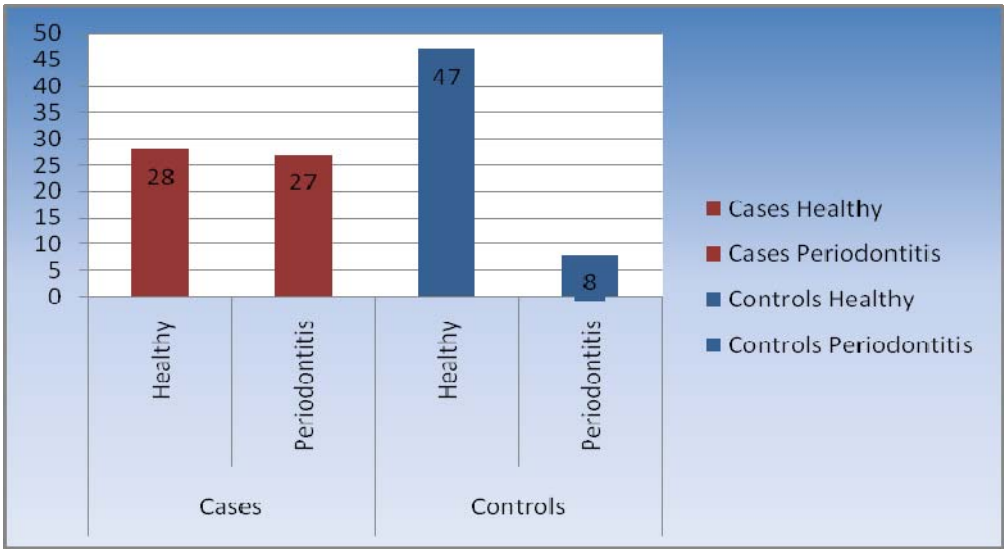


TABLE-X

COMPARISON OF RUT (P) AND CULTURE (P) BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
RUT P	No	7	12.7	26	47.3	<0.0001 (Sig.)
	Yes	48	87.3	29	52.7	
Culture P	Negative	55	100.0	55	100.0	-
	Positive	0	0.0	0	0.0	

FIGURE VI

COMPARISON OF RUT (P) BETWEEN CASES AND CONTROLS

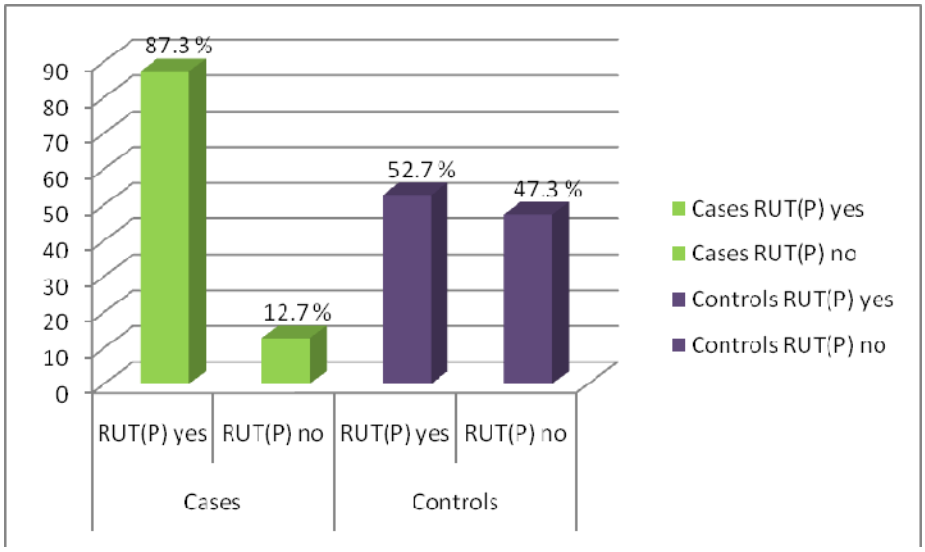


TABLE-XI

COMPARISON OF RUT (B), CULTURE (B), GRAM STAIN AND GIEMSA STAIN BETWEEN CASES AND CONTROLS

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
RUT B	No	0	0.0	55	100.0	<0.0001 (Sig.)
	Yes	55	100.0	0	0.0	
	Positive	0	0.0	0	0.0	
Culture B	Negative	55	100.0	55	100.0	<0.0001 (Sig)
	Positive	2	0.0	0	1.8	
Gram Stain	Negative	15	27.3	55	100.0	<0.0001 (Sig.)
	Positive	40	72.7	0	0.0	
Giemsa stain	Negative	5	9.1	55	100.0	<0.0001 (Sig.)
	Positive	50	90.9	0	0.0	

TABLE XII

RESULTS OF MULTIPLE LOGISTIC REGRESSION ANALYSIS

Independent Variable	Odds Ratio [95% CI]	P-value*
Contact with animals	6.13 [1.70 to 22.14]	0.006 (Sig.)
Periodontal disease Status	2.89 [1.06 to 7.89]	0.04 (Sig.)
RUT P	5.05 [1.71 to 14.89]	0.003 (Sig.)

TABLE-XIII

ASSOCIATION OF OHI AND PERIODONTAL DISEASE WITH RUT P IN CASES

Variable	Category	RUT P - No [n=7]		RUT P - Yes [n=48]		P – value*
		No.	%	No.	%	
OHI-SCORE	Poor	1	14.3	10	20.8	0.40 (N.S.)
	Fair	2	28.6	23	47.9	
	Good	4	57.1	15	31.3	
Periodontal disease	Healthy	4	57.1	24	50.0	1.00** (N.S.)
	Periodontitis	0	0.0	27	53.8	

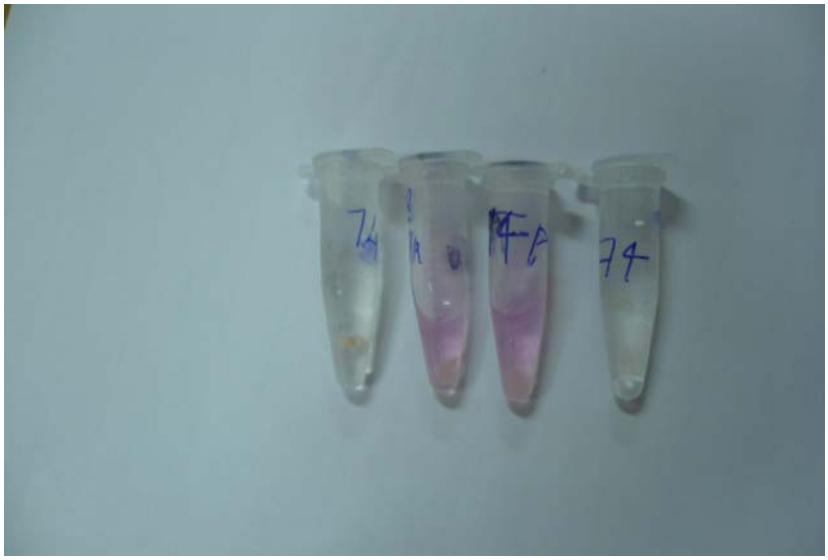
* Pearson's Chi-square test was used to calculate the P-value.
** Fisher's Exact Test (2 –tailed) was used to calculate the P-value.

TABLE-XIV

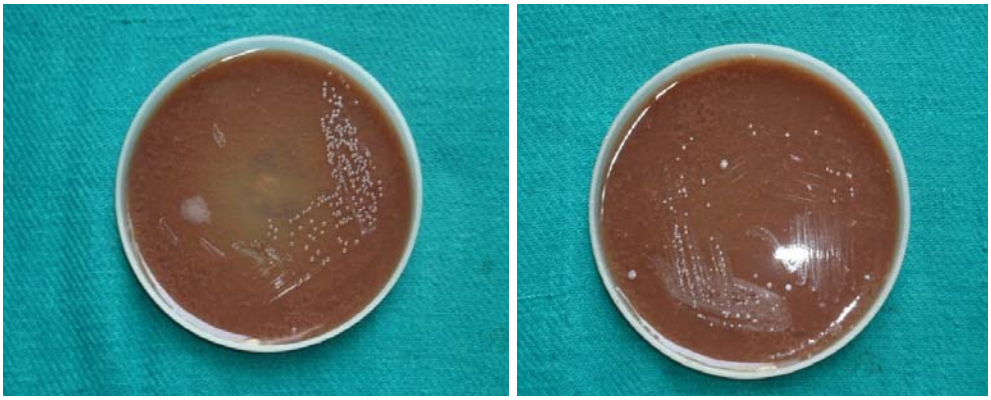
ASSOCIATION OF OHI AND PERIODONTAL DISEASE WITH RUT P IN CONTROLS

Variable	Category	RUT P - No [n=26]		RUT P - Yes [n=29]		P – value*
		No.	%	No.	%	
OHI-SCORE	Poor	4	15.4	6	20.7	0.63 (N.S.)
	Fair	15	57.7	13	44.8	
	Good	7	26.9	10	34.5	
Periodontal disease	Healthy	26	100.0	21	72.4	0.005** (Sig.)
	Periodontitis	0	0.0	8	27.6	

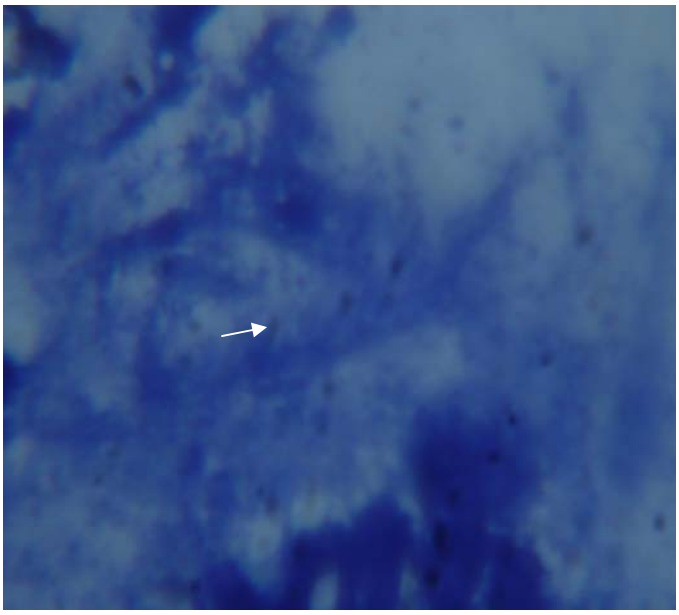
* Pearson's Chi-square test was used to calculate the P-value.
** Fisher's Exact Test (2 –tailed) was used to calculate the P-value.



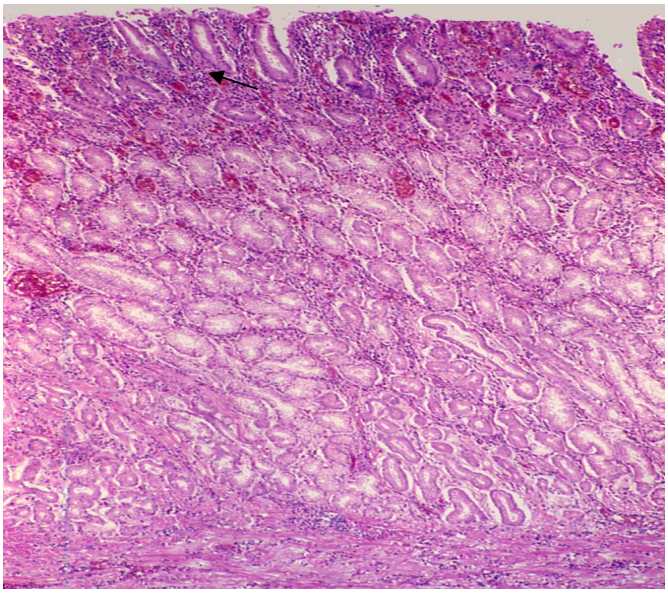
Photograph No.19: RUT positive in dental plaque and antral biopsy samples



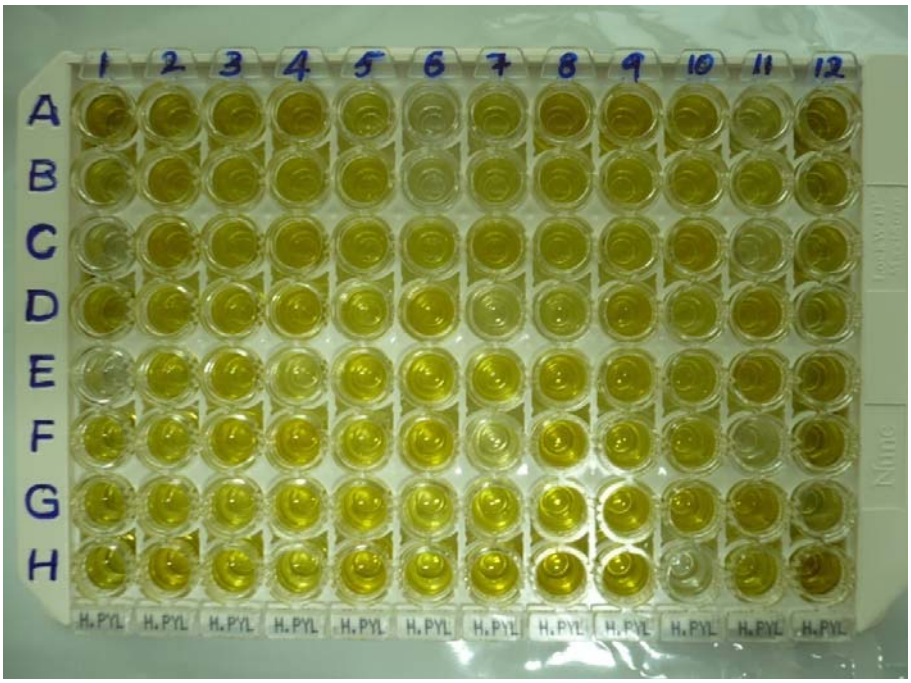
Photograph No. 20: Culture positive for H.pylori in antral biopsy



Photograph No. 21: Giemsa stain showing H.pylori in antral biopsy specimen



Photograph No. 22: Histopathological features of chronic gastritis showing chronic lymphocytic infiltration



Photograph No.23: Microplate wells after ELISA reaction

Photograph No. 24: Microplate wells in ELISA reader

DISCUSSION

Periodontal disease may be related to a number of systemic diseases including increased incidence of atherosclerosis, coronary heart disease, stroke, diabetes mellitus, pre-term low birth weight delivery and respiratory diseases (***Mealey BL 1999)***[76]**.**

Robinson and Moynihan in 1904[74] suggested that oral sepsis might play a part in the pathogenesis of gastric ulcers. The discovery of Helicobacter pylori and the acceptance of its role in gastric pathophysiology represented a fundamental change in the understanding of gastro duodenal disease (***Martin J.M. Buckly and Colm A.O' Morain 1998)***.[73] It is now acknowledged worldwide that type B gastritis, duodenal ulceration, gastric ulceration, gastric adenocarcinoma and gastric mucosa associated lymphoid tissue lymphoma are in part infectious diseases.[73] Antibiotic based regimens are now the recommended treatment for peptic ulceration.[73]

Recently attention has been focused on the importance of dental plaque in harbouring H.pylori and its role in the epidemiology of H.pylori infection.

Dental plaque has been defined as the diverse community of micro-organisms found on the tooth surface as a biofilm embedded in an extracellular matrix of polymers of host and microbial origin (***Marsh P.D. 2004).***[68] Dental plaque typically adheres to supragingival and subgingival tooth surfaces and it will quickly form in the absence of good oral hygiene measures.

Chronic periodontitis is the most prevalent form of periodontitis and occurs in response to chronic plaque and calculus accumulation (***Carranza).***[87]

This study was undertaken i) to determine whether dental plaque harbors H.pylori and may act as a reservoir for the organism and ii) to determine the relation between oral hygiene status, periodontal disease status and H.pylori gastritis.

Patients with active bleeding ulcers were excluded from the study, because the rapid urease test (RUT) lacks sensitivity in Helicobacter pylori diagnosis when peptic ulcer disease presents with bleeding (***Lee J. M. et al 2000)***.[56] Thus patients on chronic use of NSAIDS were also excluded, as NSAIDs are related to increased incidence of bleeding ulcers (***Francis Megraud and Philippe Lehours 2007).***[35]

The major period of H.pylori acquisition is in childhood (***Roger. A. Feldman et al 1998)***.[99] Once H.pylori is acquired, it persists into old age, unless eradicated by treatment (***Dowsett. S.A. M.J. Kowolik 2003)***.[27] Hence the age group in the present study had a wide range of 17-76 years.

Seroprevalence of H.pylori infection is often similar in males and females(***Gasbarrini G. et al 1995)***.[38] The gender distribution of cases and controls in the present study was similar indicating that there was little influence of these variables on the disease status of the two groups (Table III).

Lower socioeconomic status and or a low level of education are associated with an increase in the prevalence of H.pylori infection

(***Malathy HM et al 1996)***.[63]As the socioeconomic status of individuals and countries has risen, the prevalence in younger generations has declined (***Haruma et al 1997)***.[45] However in developing countries, socioeconomic status and sanitary conditions have improved even more slowly, which is thought to account for the continuing high rates of infection in young people. In the present study although there were more subjects of lower socioeconomic status, there was no significant difference in the mean SES score between cases and controls (Tables V and VI).

Handling of animals by the subjects in the two groups was also evaluated (Table IV). The proportion of subjects who had contact with animals among cases (36.4%) is significantly higher than among controls (7.3%), with a P value of 0.001 and an odds ratio of 6.13 (by multiple logistic regression analysis) 95% CI: 1.70 to 22.14 (Table XII) . Animals have been shown to harbor H.pylori. Cats are found with H.pylori in nature and could be a reservoir for human infection (***Fox JG. 1995)***.[34] In the present study, the subjects had contact with animals like cats, dogs, hens, goats and cows. However the type of animal the subjects had contact with was not statistically significant, P – 0.07 (Table IV). This may be due to the small sample size and studies on larger population may help identify the animal with high risk for acquiring H.pylori.

The high degree of association between handling of animals and H.pylori infection in the present study, suggests that animals may play an important role in the transmission of H.pylori infection and that handling of animals may be an important risk factor for developing H.pylori infection.

The oral hygiene status of patients was examined using the Simplified Oral Hygiene Index of Greene and Vermilion 1964 in the present study. The mean OHI score in cases is higher than controls. However there is no significant difference in OHI score between cases and controls (P = 0.75) (Table V). This is similar to the study by ***Pradeep et al 2006,***[94] wherein the OHI status between cases (n = 65) and controls (n = 69) was not statistically significant. In the present study, there were more subjects with good oral hygiene (19 – cases and 17 controls) than subjects with poor oral hygiene (11 – cases and 10 controls) respectively (Table VIII). This may be because there were more females (39 female patients among cases & controls) (Table III). Men have poorer oral hygiene than women, as evidenced by higher levels of plaque & calculus (***U.S. Public Health Service, National Institute of Dental Research 1987).***[108]

In the present study H. pylori was detected in dental plaque by rapid urease test in patients with good and poor oral hygiene among cases and controls.

H. pylori may be part of normal oral microenvironment and belong to a normal bacterial film that is not a pathogenic reservoir of H. pylori for the stomach. However when the host's immunological defense becomes impaired, bacteria's role as commensal is changed and it becomes a pathogen (***M. Czenikiewicz – Guzik et al 2005)***.[21]

H.pylori could be a transient microbe which comes in with food and vanishes after some time depending on some host factors as well as its interactions with the residual flora of oral biofilm (***Okuda et al 2000)***.[89]

Apart from the oral-oral & faecal-oral route of transmission, another proposed route of transmission of H. pylori is gastro – oral (***Axon ATR 1995)***.[6] Gastritis especially the acute stage is often accompanied by increased episodes of intermittent reflux which may facilitate the passage of viable organisms into the mouth (***Axon ATR 1995)***.[6] Given the forcible nature of emesis, plaque biofilm could become impregnated with ejected H. pylori,(***Parsonnet 1999)***[93] and may serve as a source of infection or reinfection.

The periodontal disease status of the patient was also examined. The diagnosis of chronic periodontitis was based on Armitage classification[2] 1999. Patients with generalized and localized periodontitis were put together and grouped as those with periodontitis and the remaining were grouped as healthy. Pocket ≥ 5mm in at least one site was associated with increased odds of H.pylori seropositivity in the NHANES Survey (1988-1991). Hence this was also considered as a variable in the present study (Table IX).In the present study there were more subjects with periodontitis among cases than controls. This was found to be statistically significant with a P value of < 0.001 and OR of 2.89 (95% CI 1.06 to 78) by multiple logistic regression analysis (Tables VII and X).

It concurs with the study by ***B.A. Dye et al 2002***,[29] that poor periodontal health characterized by advanced periodontal pockets may be associated with H. pylori infection in adult patients independent of poverty status.

H.pylori survives in moderate to advanced periodontal pockets because the architecture and the microcosm of these periodontal

conditions promote a viable habitat for microaerophilic and anaerobic micro-organisms. Because dental biofilm can provide urea, urease producing bacteria such as H.pylori may have improved viability in this periodontal environment.

Correlation analysis was done between oral hygiene status of the patients and presence of H. pylori in dental plaque and thus with gastric infection in cases and controls. It was not found to be statistically significant (Table XIII and XIV).The reasons are as given above.

When the periodontal disease status was correlated with oral H.pylori and the corresponding gastric infection in cases and controls, it was found that although all the cases of periodontitis were positive for H.pylori by RUT, it was not statistically significant (P=1.00) in cases but was found to be statistically significant (P=0.005) in controls(Table XIII & XIV).In certain oral conditions like periodontitis, mucosal ulcers, the numbers of H.pylori may increase and reach levels sufficient to cause infection, especially when the immunological status of the patient is compromised ***Choudary CR et al 2003***.[19]Hence the periodontal pocket could serve as a reservoir for future gastric infection in these controls.

The methods of detection of H.pylori in dental plaque vary and include the rapid urease test, culture and PCR. In the present study, both the rapid urease test and culture were employed to detect the presence of H.pylori in dental plaque. H. pylori is the only urease – positive bacteria known to reside in the stomach; hence the rapid urease test (RUT) can be used to confirm the presence of H. pylori in gastric samples.

The oral cavity is residence to several urease producing species including Streptococcus species, Haemophilus species and Actinomyces species. But ***Vaira D.1988***[109] reported that urease positive micro-organisms present in the oral cavity usually cannot give positive results within an hour. Hence the RUT has been used by several studies to detect the presence of H.pylori in dental plaque ***Gill et al 1994***[39]***, Pytko Polonczyk et al 1996***[96]***, Desai et al 1998***[25]***, Butt et al 1999***[17]***, Avcu et al 2001***[5]***, Pradeep et al 2006.***[94] Studies have reported that the RUT has a specificity near 100% and sensitivity between 70% and 93% (***Mc Nulty 1992***[75]***, Delterse M. 1988)***.[24] The sensitivity of using the RUT in dental plaque to determine H.pylori status is reported to be 89.7% with a diagnostic accuracy of 86.7% (***Gurbuz et al 2003)***.[43]

In the present study, more cases (48 of 55) had positive RUT in the dental plaque than controls (29 of 55) and this was found to be statistically significant with a P value of < 0.001 and OR of 5.05 with 95% CI of 1.71 to 14.89 (by multiple logistic regression analysis) (Tables X and XII).This is similar to the study by ***Pradeep et al 2006***, [94] wherein 58 of 65 cases and 49 of 69 controls were RUT positive in dental plaque. This was found to be statistically significant with an OR of 3.10 (95% CI 1.21 to 9.77).

In the present study, H.pylori could not be detected by culture in dental plaque samples of either cases or controls (Table X). This is in accordance with other studies, wherein there was no cultural detection of H.pylori in dental plaque samples- ***Cheng et al 1996 0/122***[18] dental plaque samples***, Luman et al 1996 0/109***[59]***, Allaker et al 2002 0/100***[1]***,***

Ishihara et al 1997 0/87[48]***, Hardo et al 1995 0/65***[44] dental plaque samples.

The failure of culture methods to detect H.pylori from dental plaque may be due to the following reasons

i) Insufficient numbers in oral samples(***Song et al 2000).***[103]

ii) H.pylori requires a microaerophilic environment, supplemented media and up to 7 days incubation for growth. Under these conditions, overgrowth by other oral species is likely and may inhibit the growth of oral H.pylori (***Ishihara et al 1997).***[48]

iii) Absence of culturable oral H.pylori and the presence of a viable coccoid form that is unculturable by conventional techniques (***Bode et al 1993).***[16]

iv)Being microaerophilic, these bacteria may not able to survive the sampling process before being transferred into the medium. This is particularly true, when the numbers of colonizing bacteria are low ***(Asikainen S. et al 1994)***[4]**.**

The results of this study showed that although the rapid urease test was positive for 48 of 55 cases and 29 of 55 controls, culture was negative in the dental plaque samples of all cases and controls. Hence the role of dental plaque as a reservoir for H.pylori infection is inconclusive.

SUMMARY AND CONCLUSION

110 patients with dyspeptic symptoms and clinical indications for an upper gastroendoscopy from the outpatient Department of Medical Gastroenterology Government General Hospital Chennai were selected for the study. The study variables age, gender, handling of animals, smoking and alcohol consumption were obtained from the patient's history. The socio-economic status was arrived, based on Kuppuswamy's Socio-economic status 2007. Oral hygiene status was evaluated using the Simplified Oral Hygiene Index of Greene and Vermillion 1964. Probing depth and clinical attachment level were recorded and based on the AAP 1999 classification , patients were classified into periodontitis and healthy patients. Antral biopsy samples (during endoscopy) and dental plaque samples were obtained. H.pylori in dental plaque was detected by RUT and culture, while antral biopsy samples were examined for the presence of H.pylori by RUT, culture, histopathology and ELISA. Patients with clinical symptoms and a positive test on antral biopsy sample by any of the three diagnostic tests – RUT, histology, and serology were cases and those negative were controls.

Among the variables, handling of animals was found to be a risk factor for acquiring H.pylori infection. The oral hygiene status of the patients did not influence H.pylori infection. Patients with periodontitis were at a higher risk for developing H.pylori infection and pocket depths ≥5mm could serve as a reservoir for the organism. All these observations are based on the positive results obtained with the rapid urease test in

dental plaque. However there was no cultural detection of H.pylori in dental plaque samples in this study. Hence the presence of H.pylori in dental plaque is inconclusive.

Dental plaque cannot however be discounted as a possible alternate site for the organism. The present methods may be inadequate to reliably isolate the organism from this site. A more comprehensive search for the organism in this environment and other ecological niches within the gingival crevices ought to be conducted to elucidate the role of dental plaque as a potential reservoir for H.pylori.

If the oral cavity is a reservoir for gastric infection, even in a minority of individuals, the control of dental plaque along with standard periodontal procedures should be recommended for patients with chronic gastritis or peptic ulcer.

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APPENDIX
PROFORMA

ASSOCIATION OF DENTAL PLAQUE, POOR ORAL HYGIENE AND PERIODONTAL DISEASE WITH HELICOBACTER PYLORI INFECTION.

Date:

O.P.No:

Name:

Code No:

Age/Sex:

Address: Tel.No:

Occupation: Income:

Level of education: SES score:

Chief complaints:

Duration:

History:

Smoker – Current/Ex-smoker/ Not at all

Alcohol– Current/Past/ Never

H/o contact with animals- Yes/No

Type of animals-

SIMPLIFIED ORAL HYGIENE INDEX- GREENE AND VERMILION 1964

DEBRIS INDEX

16	**11**	**26**
46	**31**	**36**

CALCULUS INDEX

16	**11**	**26**
46	**31**	**36**

DI = Sum of debris score per tooth surface / No. of surfaces examined

CI = Sum of calculus score per tooth surface / No. of surfaces examined

OHI score= Debris index score+ Calculus index score
Interpretation- Good/Fair/ Poor oral hygiene

PROBING DEPTH (PPD) AND CLINICAL ATTACHMENT LOSS (CAL) (in mm) (CARRANZA)

MAXILLARY **Buccal**

CAL															
PD															
	18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
PD															
CAL															

Palatal

MANDIBULAR **Buccal**

CAL															
PD															
	48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
PD															
CAL															

Lingual

Periodontal disease status – H- Healthy G- Generalized chronic periodontitis L- Localized chronic periodontitis

RUT (P):

RUT (B):

CULTURE (P):

CULTURE (B):

GIEMSA STAIN:

GRAM STAIN:

ELISA:

HISTOPATHOLOGICAL FINDINGS:

INFORMED CONSENT FORM-ENGLISH

Study Title:

ASSOCIATION OF DENTAL PLAQUE, POOR ORAL HYGIENE AND PERIODONTAL DISEASE WITH HELICOBACTER PYLORI INFECTION

Date:	O.P. No:
Name:	Code No:
Address:	Age/Sex:
	Tel. No:

I, age yrs, exercising my free power of choice, hereby give my consent to be included as a participant in the clinical study "Association of dental plaque, poor oral hygiene and periodontal disease with Helicobacter pylori infection".

I agree to the following:

I have been informed to my satisfaction about the purpose of the study, and nature of the study.

I understand that I should undergo endoscopy procedure as a part of diagnosis for my condition in the stomach. The tissue sample taken during the procedure for the purpose of diagnosis will be used for study purpose and helps in confirming the diagnosis.

I understand that the investigations will also require oral plaque sample in required amounts.

I agree to co-operate fully and participate in the study.

I hereby give permission to use the records for study. I am told that study doctor and study institution will keep my identity confidential.

Signature of the Investigator	Signature of the Guide	Signature of the Participant

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு:

”எச்.பைலோரி நுண்ணுயிர் தொற்றுள்ள நிலையில் பற்புறத்திசு நோய்க்கும், பற்கிருமி படலம் மற்றும் பராமரிக்கப்படாத வாய்சுகாதாரம் குறித்து தொடர்பு”.

தேதி : ***புறநோயாளி எண் :***
பெயர் : ***ஆராய்ச்சி சேர்க்கை எண் :***
முகவரி : ***வயது : ஆ/பெ:***
தொலைபேசி எண் :

நான் _____ வயது _____ என்னுடைய சுயநினைவுடன் மற்று முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கபட்ட விஷயங்களுக்கு நான் எனது சம்மதத்தை தருகிறேன்

- இந்த ஆராய்ச்சியின் நோக்கம் மருத்துவ முறைகள் பரிசோதனை முறைகள் எனக்கு திருப்தியுறும் வகையில் விளக்கப்பட்டன.

- நோய் அறியும் செயலின் ஒரு பகுதியாக எண்டோஸ்கோபி சோதனைக்கு நான் உட்பட வேண்டும் என்பதை உணர்ந்து கொண்டேன். மேலும் மேற்கண்ட சோதனையின் திசுப்பரிசோதனைக்காக சிறிய அளவில் திசு எடுக்கப்படும் எனவும் அது நோயை அறிய உதவும் எனவும், எந்த விதத்திலும் அது சிகிச்சையை பாதிக்காது எனவும் உணர்ந்து கொண்டேன். மேலும் வழக்கமாக செய்யும் இரத்தப் பரிசோதனையும் இந்த ஆய்வுக்கு உபயோகப்படுத்தப்படுகிறது.

- கிருமியை பரிசோதனை செய்வதற்காக பற்களில் படிந்திருக்கும் பற்படலத்தில் சிறிதளவு எடுக்க வேண்டியுள்ளதாக அறிகிறேன்.

- எனது மருத்துவ குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக் கொள்ள சம்மதிக்கிறேன். ஆராய்ச்சி மையமும் ஆராய்ச்சியாளரும் என்னுடைய பெயர் மற்றும் சில விபரங்களை இரகசியமாக வைப்பதாக அறிகிறேன்.

பேராசியரின் கையொப்பம் ***ஆராய்ச்சியாளரின் கையொப்பம்*** ***நோயாளின் கையொப்பம்/கைநாட்டு***